

Monoclonal Anti-Alpha-Synuclein Antibodies for Preventing Tau Aggregation

Field of invention

- 5 **[0001]** The present invention relates to a novel use of monoclonal antibody that immunospecifically bind to alpha-synuclein, as well as to methods of using these molecules and their alpha-synuclein binding fragments in the treatment of tau-pathies such as Alzheimers disease (AD).

10 **Reference To Sequence Listing**

- [0002]** This application includes one or more Sequence Listings pursuant to 37 C.F.R. 1.821 *et seq.*, which are disclosed in both paper and computer-readable media, and which paper and computer-readable disclosures are herein incorporated by reference in their entireties.

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Background of the invention

- [0003]** Age-related neurodegenerative diseases such as Alzheimer's disease (AD) and dementia are one of the largest societal challenges today. The World Health Organization estimates that costs for care of the elderly will continue to increase and that the number of diagnosed dementia cases will triple by 2050 (World Health Organization and Alzheimer's Disease International - Status Report (2012) DEMENTIA: A public health priority, WHO). The first treatments for AD were neurotransmitter modulators such as acetylcholine esterase inhibitors and NMDA modulators. These therapies became available at the turn of the millennium and still form the cornerstone for symptomatic relief of memory deficits related to dementia and AD. However, these drugs do not target the underlying causes of AD, accumulation of amyloid- β (A β) peptide and tau protein aggregates and associated loss of neuronal synapses and eventually neurons.
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- 30 **[0004]** Longitudinal, community-wide studies of the elderly (Weiner, M.W. *et al.* (2014) ADNI; Breteler, M.M. *et al.* (1992) Neuroepidemiology 11 Suppl 1, 23-28; Launer, L.J. (1992) Neuroepidemiology 11 Suppl 1, 2-13) together with

large genome-wide association studies (Lambert, J.C. *et al.* (2013) *Nat. Genet.* 45, 1452-1458) have shown that AD is a heterogeneous mix of dementias where up to 10 percent of the advanced AD patients lack amyloid pathology (Crary, J.F. *et al.* (2014) *Acta Neuropathol.* 128, 755-766). Furthermore, seminal pathological studies by Braak & Braak (Braak, H. and Braak, E. (1996) *Acta Neurol. Scand. Suppl* 165, 3-12) demonstrated a clear correlation between the degree of neurofibrillary tangle pathology and cognitive state prior to autopsy. These observations have been reinforced by several investigators (Nelson, P.T. *et al.* (2012) *J. Neuropathol. Exp. Neurol.* 71, 362-381), and in recent longitudinal biomarker studies, which indicate that cerebrospinal fluid (CSF) levels of tau and phospho-tau increase throughout early and late stages of the disease (Jack, C.R., Jr. *et al.* (2013) *Lancet Neurol.* 12, 207-216).

[0005] As indicated above, the microtubule-associated protein, tau, and its hyper-phosphorylated version, form the main constituent of intracellular neurofibrillary tangles, which are one of the main hallmarks of AD. Furthermore, specific genetic variants of tau are associated with familial forms of frontotemporal dementia (FTD). Appearance of tau pathology in AD occurs in a distinct spatial pattern, starting in the entorhinal cortex, followed by hippocampal and cortical areas (Braak, H. and Braak, E. (1996) *Acta Neurol. Scand. Suppl* 165, 3-12). The specific stage of tau pathology also correlates well with cognitive abilities (Nelson, P.T. *et al.* (2012) *J. Neuropathol. Exp. Neurol.* 71, 362-381; Braak, E. *et al.* (1999) *Eur. Arch. Psychiatry Clin. Neurosci.* 249 Suppl 3, 14-22). Taken together, this evidence forms the basis of a tau-based hypothesis for AD. It entails that the intracellular accumulation of tau leads to microtubule degeneration and spinal collapse. As a result, communication between neurons malfunctions and cell death follows. Recently, it has also been shown that tau itself may form an endo-pathogenic species that can transmit neurodegeneration from one cell to the next (Clavaguera, F. *et al.* (2009) *Nat. Cell Biol.* 11, 909-913).

Tau As An Endo-Pathogen

[0006] Clavaguera and colleagues have demonstrated that tau itself may act as an endo-pathogen (Clavaguera, F. *et al.* (2009) Nat. Cell Biol. 11, 909-913). Low spin brain extracts were isolated from P301S tau transgenic mice (Allen, B. *et al.* (2002) J. Neurosci. 22, 9340-9351), diluted and injected into the hippocampus and cortical areas of young ALZ17 mice. The ALZ17 mouse is a tau transgenic mouse line which only develops late pathology (Probst, A. *et al.* (2000) Acta Neuropathol. 99, 469-481). The injected ALZ17 mice quickly developed solid filamentous pathology, and administration of immunodepleted brain extracts from P301S mice or extracts from wild type mice did not induce tau pathology. Fractionation of the brain extracts in soluble (S1) and sarcosyl-insoluble tau (P3) (Sahara, N. *et al.* (2013) J. Alzheimer's. Dis. 33, 249-263) and injection of these into ALZ17 mice demonstrated that the P3 fraction is most competent in inducing pathology. It contains most of the intracellular hyper-phosphorylated filamentous tau. The majority of pathology could also be induced when injecting P301S extracts into the brains of wild type mice, but no NFTs were formed. In subsequent studies, Clavaguera *et al.* have shown that human tau extracted from post-mortem brain tissue of other tauopathies (Argyrophilic Grain Disease (AGD), Progressive Supranuclear Palsy (PSP), and Corticobasal Degeneration (CBD)) may also induce tau pathology in the ALZ17 model (Clavaguera, F. *et al.* (2013) Proc. Natl. Acad. Sci. U.S.A. 110, 9535-9540). Since the presentation of these data, several other tau seeding and spreading models have been reported (Ahmed, Z. *et al.* (2014) Acta Neuropathol. 127, 667-683; Walker, L.C. *et al.* (2013) JAMA Neurol. 70, 304-310). The main conclusion from these studies indicates a mechanism by which pathogenic tau in intracellular inclusions is secreted from the cell into the periplasmic space. The pathological tau material is then transported along the vesicular sheath in both anterograde and retrograde direction and subsequently taken up by neighboring cells by means of bulk endocytosis. This mechanism explains why the spread of pathology observed in human disease follows a distinct anatomical pattern. Intriguingly, peripheral

administration of pathological tau may accelerate the formation of tau pathology in ALZ17 mice (Clavaguera, F. *et al.* (2014) *Acta Neuropathol.* 127, 299-301).

5 **Relation between Alpha-synuclein and Tau pathology**

- [0007] Alpha-synuclein is a member of a family of proteins including beta- and gamma-synuclein and synoretin. Alpha-synuclein is expressed in the normal state associated with synapses and is believed to play a role in regulating synaptic vesicle release and thereby affect neural plasticity, learning and memory.
- [0008] Several studies have implicated alpha-synuclein with a central role in Parkinson's Disease (PD) pathogenesis. The protein can aggregate to form intracellular insoluble fibrils in pathological conditions. For example, synuclein accumulates in Lewy Bodies (LB) (Spillantini *et al.*, *Nature* (1997) 388:839-40; Takeda *et al.*, *J. Pathol.* (1998) 152:367-72; Wakabayashi *et al.*, *Neurosci. Lett.* (1997) 239:45-8). Mutations in the alpha-synuclein gene as well as duplications and triplications of the gene co-segregate with rare familial forms of parkinsonism (Kruger *et al.*, *Nature Gen.* (1998) 18:106-8; Polymeropoulos, *et al.*, *Science* (1997) 276:2045-7).
- [0009] An important finding has been that alpha-synuclein can be secreted into and be present in plasma and cerebrospinal fluid (CSF). Several studies, for example by Pacheco *et al.* (2015) and others (Conway *et al.*, (2000) *Proc Natl Acad Sci USA*, 97:571-576; Volles *et al.*, *J. Biochem.* (2003) 42:7871-7878) have suggested that extracellular-synuclein plays a pathogenic role in the brain. They demonstrated that alpha-synuclein possesses neurotoxicity toward brain neuronal plasma membranes exposed directly to extracellular-synuclein oligomers. Another intriguing hypothesis based on the data of synuclein secretion is that a prion-like spread of alpha-synuclein underlies the progression of Parkinson's disease and other synucleinopathies (Lee *et al.*, Hansen *et al.* (2011) *J. Clin Invest* 121:715-725). These finding have given rise to a hope that extracellular-synuclein could be targeted by immunotherapy (Vekrellis *et al.* (2011) *Lancet Neurol* 10:1015-1025) and be a potential treatment of alpha-

synucleinopathies. In addition to mutations, alternative splicing of the alpha-synuclein gene and posttranslational modifications of the protein, such as phosphorylation, ubiquitination, nitration, and truncation can create alpha-synuclein protein forms that have enhanced capacity to form aggregated and/or toxic forms of alpha-synuclein (Beyer and Ariza, Mol Neurobiol. 2013 Apr;47(2):509-24). However, the precise pathological species of alpha-synuclein in alpha-synucleinopathies remains unknown. Various misfolded/aggregated/secreted species ranging from oligomers to fibrils, and different post-translational modifications have been associated with toxicity but there is no consensus on which is most important, if indeed there even is a single toxic species.

[00010] The co-appearance of pathologies, for example Lewy bodies, Abeta plaques and neurofibrillary tangles in subsets of patients with PD or Lewy bodies in a subset of AD patients (Galpern and Lang, Ann. Neurol. (2008), 59: 449-458) has led to investigations of to what extent aggregation prone proteins can cross-seed each other. Alpha-synuclein and tau proteins have been reported to be able to induce fibrillization of each upon co-incubation in vitro (Giasson et al., (2003) Science 300: 636-640). In cellular systems there is both evidence supporting and not supporting a cross-seeding of tau with alpha-synuclein fibrils. Holmes et al., could not demonstrate a cross-seeding of tau with fibrils made from full-length alpha-synuclein in a FRET based tau reporter cell line (Holmes et al., (2014) PNAS doi/10.1073: E4376-E4385). Nor could Tau aggregation be induced with fibrillated full-length alpha-synuclein A53T or a PTA-precipitated (to enrich for fibrillated alpha-synuclein) brain sample from a multiple system atrophy (MSA) patient (Woerman et al., (2015) PNAS doi/10.1073: E4949-E4958. Others have reported that under some conditions alpha-synuclein can induce tau aggregation, for example alpha-synuclein fibrils made from N-terminal truncated (21-140) alpha-synuclein was shown to induce tau phosphorylation in QBI293 cells (Waxman and Giasson (2011) J. Neurosci 31: 7604-7618). In neuronal cultures full length fibrillated alpha-synuclein do seed tau aggregation. However fibrillated alpha-synuclein made from truncated alpha-synuclein (1-120 or 32-140) can through repeated self-seeding in cells (5 or 10% of fibrillated alpha-synuclein

from each passage included as seeds in the fibrilization of the subsequent passage) (Guo et al., (2013) Cell 154: 109-117).

[00011] To our knowledge no studies has hypothesised that secreted oligomeric or fibrillated forms of alpha-synuclein could be a contributing factor in the early pathogenesis of AD or other tauopathies in general independent of visible alpha-synuclein inclusions. The reports that could demonstrate a cross-seeding of tau with alpha-synuclein have focussed on explaining why there in some PD patients, with manifest alpha-synuclein aggregation (Lewy bodies) are found neurofibrillary tangles. These studies speculate that the cross-seeding can take place in areas where there is a close physiological association of tau and alpha-synuclein deposits (Giasson et al., (2003) Science 300: 636-640; Waxman and Giasson (2011) J. Neurosci 31: 7604-7618; Guo et al., (2013) Cell 154: 109-117). The idea that soluble extracellular oligomeric/fibrillated forms of alpha-synuclein and not intracellular aggregates in the form of Lewy bodies or Lewy neurites are the important contributing factor in the generation of tau neurofibrillar tangles is new.

Alpha-synuclein immunotherapies

[00012] Antibodies binding to alpha-synuclein have been developed as potential therapeutic agents to treat synucleinopathies, also known as Lewy body diseases (LBDs). Synucleinopathies are characterized by deposition of intracellular protein aggregates microscopically visible as Lewy bodies (LBs) and/or Lewy neurites, where the protein alpha-synuclein is a major component (Jellinger, Mov Disord. 2012 Jan;27(1):8-30; McKeith et al., Neurology (1996) 47:1113-24). Synucleinopathies include Parkinson's disease (including idiopathic Parkinson's disease) and Diffuse Lewy Body (DLB) disease (also known as Dementia with Lewy Bodies (DLB), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson disease (PD), pure autonomic failure and multiple system atrophy (MSA; e.g., Olivopontocerebellar Atrophy, Striatonigral Degeneration and Shy-Drager Syndrome).

[00013] Several different antibodies to alpha-synuclein have been shown to have therapeutic effect in preclinical animal models. Both an antibody targeting an epitope involving alpha-synuclein residues 91-99 and antibodies targeting an

epitope that involves alpha-synuclein residues 118-126 have been shown to have an effect on motor and cognitive deficits in transgenic mice (Games et al. 2014). The most advanced of these antibodies is a humanized antibody based on the mouse monoclonal antibody 9E4, which targets an epitope that involves alpha-synuclein residues 118-126, and which is now in clinical trials in phase I. Also an antibody that targets an amino-terminal epitope of alpha-synuclein has been shown to have possible therapeutic potential in preventing spreading and toxicity of pathology in a mouse prion like transfer model (Tran et al. 2014) and a C-terminal antibody 274 which targets an epitope that involves alpha-synuclein residues 120-140 (Bae et al. 2012) was also shown to have an effect in preclinical model on spreading of the pathology from cell to cell. In addition to these, antibodies targeting conformational species such as oligomers and fibrils of alpha-synuclein have been shown to be able to at least reduce the levels of these presumably toxic alpha-synuclein species (Lindtsröm et al. 2014 and Spencer et al. 2014). These conformational antibodies that lower alpha-synuclein oligomer levels in vivo, such as mab47 were also shown to target epitopes in the C-terminus of alpha-synuclein, from amino acid 121-125 (US20120308572). Other conformational, fibril and oligomer specific antibodies also target C-terminal sequences (Vaikath et al. Neurobiol Dis. 2015 Apr 30;79:81-99). Importantly none of these alpha-synuclein antibodies has been claimed to be able to prevent tau aggregation and as a consequence be able to potentially treat tauopathies.

[00014] In this invention we surprisingly discovered that aggregated/fibrillated alpha-synuclein can induce aggregation of Tau and that several antibodies generated to bind alpha-synuclein are able to prevent this aggregation. We show that a panel of different alpha-synuclein antibodies are all able to prevent aggregation of Tau in the cellular model: An antibody (GM37) that can bind to the presumed toxic alpha-synuclein fragment 1-119/122 (binding to amino acids 112-117 of alpha-synuclein) and neutralize this truncated form of alpha-synuclein, an antibody (2E6) that bind to amino acid 136-140 of alpha-synuclein, an antibody (GM63) that bind to amino acid 126-138 of alpha-synuclein and an antibody 9E4 that bind to amino acid 118-126 of alpha-synuclein. To support that fibrillary forms of alpha-synuclein may contribute

to early AD pathology we demonstrate the presence of fibrillated alpha-synuclein in brains from AD patients independent of the presence of Lewy body pathology. This supports that soluble extracellular forms of fibrillated alpha-synuclein can potentially play a role in contributing to Tau-pathology in tauopathies such as all AD patients and not only in those characterised with visible alpha-synuclein aggregates (determined by brain imaging or post mortem staining).

Summary of the invention

[00015] The present invention relates to the use of an alpha-synuclein binding monoclonal antibody for inhibiting aggregation of tau.

[00016] The antibodies of the invention, as disclosed herein and in the claims, can be used in treating patients with Alzheimer's disease or patients with a tauopathy such as Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), Picks Disease, Primary age-related tauopathy (PART), Neurofibrillary tangle-predominant senile dementia, Dementia pugilistica, Chronic traumatic encephalopathy, stroke, stroke recovery, neurodegeneration in relation to Parkinson's disease, Parkinsonism linked to chromosome, Lytico-Bodig disease (Parkinson-dementia complex of Guam), Ganglioglioma and gangliocytoma, Meningioangiomatosis, Postencephalitic parkinsonism, Subacute sclerosing panencephalitis, Huntington's disease, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease and lipofuscinosis. More typically, the tauopathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear

Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), and Picks Disease.

5 **[00016a]** In one aspect of the present disclosure, there is provided a use of an alpha-synuclein binding monoclonal antibody, or an antigen-binding fragment thereof in the manufacture of a medicament for inhibiting aggregation of tau.

10 **[00016b]** In another aspect of the present disclosure, there is provided a pharmaceutical composition comprising the monoclonal antibody, or an antigen-binding fragment thereof as described herein, and a pharmaceutical acceptable carrier.

Brief description of figures

15 **[00017]** Fig. 1 shows immunization protocols for generation of hybridomas. The table outlines the differences of the immunogens and mouse strains used for the identification of GM37 and GM285. Different HCo17-Balb/c and HCo12/Balb/c mice were immunized independently (description of these mice are provided below). The hybridoma expressing GM37 was identified from mice immunized with full length alpha-synuclein containing amino acids 1-140 fibrils and boosted with truncated alpha-synuclein fragments 1-60 and 1-119 of full length (FL) alpha-synuclein (**SEQ ID NO 10**). The hybridoma expressing antibody GM285 came from an immunization protocol in which HCo12-Balb/c mice were immunized with full length monomeric alpha-synuclein, amino acids 1-140 followed by a boost with full length fibrillary alpha-synuclein (Example 1).

25 **[00018]** Fig. 2 (PANEL A-C) shows screening of GM37 for binding to alpha synuclein, alpha-synuclein homologs and orthologs.

[00019] A) Binding of antibody GM37 to alpha-synuclein using a no wash solution based ELISA (FMAT).

[00020] B) Using SPR (Fortebio) binding of antibody GM37 is specific for alpha-synuclein (Alpha Panel) and does not bind the other related synuclein family proteins, beta-synuclein (Beta Panel) and gamma-synuclein (Gamma Panel). Measurements were performed using SPR (Fortebio Octetred) GM37 shows similar binding to alpha-synuclein from cynomolgus monkey (Cyno Panel) and mouse (Mouse Panel). (Example 1).

[00021] C) Using SPR (Fortebio Octetred) binding of antibody GM285 is specific for alpha-synuclein and does not bind the other related synuclein family proteins, beta-synuclein and gamma-synuclein. Measurements were performed

using SPR (Fortebio Octetred) shows similar binding of GM285 to alpha-synuclein from cynomolgus monkey (Cyno) and mouse (Mouse)(Example 1).

[00022] Fig. 3 (Panels A-C) shows real time binding Affinity of GM37

5 **[00023] A)** Binding of antibody GM37 to alpha-synuclein measured in RU (Relative Units) (y-axis) over time (X-axis) as determined by SPR (BIAcore® 3000). Goat anti-human IgG was immobilized on the CM5 chip. GM37 was captured on the Goat anti-human IgG immobilized chip and series of concentrations of human alpha-synuclein (3.125, 6.25, 12.5, 25, 50, 100 nM) were tested on binding to the surface. The sensor surface was regenerated between each cycle.
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[00024] B) Signal from binding at different concentrations converted into a binding curve.

[00025] C) Calculated binding constants of antibody GM37 (denoted hlgG1-6004-037-C106S) (Example 2).

15 **[00026] Fig. 4** (Panels A-C) shows real time binding Affinity of GM285

[00027] A) Binding of antibody GM285 to alpha-synuclein measured in RU (y-axis) over time (X-axis) as determined by SPR (BIAcore® 3000). Goat anti-human IgG was immobilized on the CM5 chip. GM285 was captured on the Goat anti-human IgG immobilized chip and series of concentrations of human alpha-synuclein (3.125, 6.25, 12.5, 25, 50, 100 nM) were tested on binding to the surface. The sensor surface was regenerated between each cycle.
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[00028] B) Signal from binding at different concentrations converted into a binding curve.

25 **[00029] C)** Calculated binding constants of antibody GM285 (denoted hlgG1-6004-285) (Example 2).

[00030] Fig. 5 (Panels A-C) shows real time binding of comparator antibody 9E4

- 5 **[00031]** A) Shows binding of 9E4 to alpha-synuclein measured in RU (y-axis) over time (X-axis) as determined by SPR (BIAcore® 3000). Goat anti-human IgG was immobilized on the CM5 chip. 9E4 was captured on the chip by its binding to Goat anti-human IgG that had been immobilized to the chip. A series of concentrations of human alpha-synuclein (3.125, 6.25, 12.5, 25, 50, 100 nM) were tested for binding to the surface. The sensor surface was regenerated between each cycle.
- 10 **[00032]** B) Signal from binding at different concentrations converted into a binding curve.
- 10 **[00033]** C) Calculated binding constants for antibody 9E4. (Example 2).
- 15 **[00034]** **Fig. 6** (Panels A-B) shows epitope mapping of antibody GM37 and GM285. ELISA data showing relative levels of binding of the antibodies to sequential peptides (20mers) derived from alpha-synuclein amino acid sequence 95 - 132 (the other nonbinding peptides are not shown).
- 15 **[00035]** A) GM37 epitope requires peptide sequence ILEDMP (**SEQ ID NO:9**) for full binding.
- 15 **[00036]** B) GM285 requires peptide ILED (**SEQ ID NO:19**) for full binding. (Example 3).
- 20 **[00037]** **Fig 7** shows a table comparing the binding rate kinetic parameters of GM37 and variants 1-3 to immobilized recombinant human alpha-synuclein. The binding was measured using SPR and the rates were determined using a 1:1 binding algorithm (BIAcore® T200).
- 25 **[00038]** **Fig. 8** Tau aggregation induced by alpha-synuclein seeds prevented by alpha-synuclein antibodies. Figure 7 upper panel shows that tau aggregation can be efficiently induced by alpha-synuclein seeds (fibrillated recombinant alpha-synuclein) in a type of cellular model commonly used to assess the ef-

fect of agents interfering with Tau aggregation. The alpha-synuclein seed induced tau aggregation can be prevented by alpha-synuclein antibodies in general – exemplified by 9E4 (Elan, Prothena) and Lundbeck antibodies HLD1, GM37 (“37”) and GM63 (“63”). Antibodies against Tau phosphorylated on Serine 396 (D1.2, C10.2 and humanized (h) c10.2 and another tau antibody denoted Lu0041G) and control antibodies with no affinity for alpha-synuclein have no effect on tau aggregation, supporting the importance of the therapeutic antibody to interact with the seeding species, not the endogenous protein (Example 12). Figure 8 lower panel shows an outline of the aggregation assay in HEK293 cells. Cells are transfected with cDNA encoding human full length Tau with the P301L mutation. Twentyfour hour’s later cells are treated with alpha-synuclein seeds in combination with antibodies. After 48 hours the level of Tau aggregation is measured in cell homogenates using a biochemical assay (**Example 5**).

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[00039] Fig 9 Presence of alpha-synuclein aggregates in frontal cortex from all 50 AD cases – group divided in mid-stage (Braak III/IV) and late-stage (Braak V/VI) AD. 2 DLB samples are included as control (two upper lines in A). No detection of alpha-synuclein-serine129 phosphorylation in AD samples. Dementia with Lewy body (DLB) samples are included positive as controls.

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A and B demonstrate the presence of aggregated alpha-synuclein in frontal cortex of 50 AD patients measured by a biochemical method. Patients where clinically diagnosed with AD and the diagnosis confirmed by postmortem histological staining for Tau and Abeta. None of the patients presented with Lewy body pathology (aggregated serine129 phosphorylated alpha-synuclein), figure 9C. The presence of alpha-synuclein aggregates in all patients and the absence of serine129 phosphorylated alpha-synuclein (a marker for Lewy bodies), supports the hypothesis that aggregated forms of alpha-synuclein is present in all AD patients before alpha-synuclein pathology might manifest as Lewy bodies. We suggest that these aggregated – pre-Lewy body forms of alpha-synuclein can act as a contributing factor in inducing tau pathology (figure 8). In summary we hypothesize that any alpha-synuclein antibody that are capable of neutralizing alpha-synuclein aggregates (seeds) or

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by other means prevent alpha-synuclein aggregates in entering neurons or glia cells and facilitate aggregation of Tau, will have a therapeutic potential to treat tauopathies.

5 [00040] DETAILED DESCRIPTION OF THE INVENTION

Definitions

[00041] As used herein, the term “alpha-synuclein” is synonymous with “the alpha-synuclein protein” and refers to any of the alpha-synuclein protein isoforms (identified in, for example, UniProt as P37840, 1-3). The amino acid
10 numbering of alpha-synuclein is given with respect to **SEQ ID NO:10** as shown below, with methionine (M) being amino acid residue1:

SEQ ID NO:10:

MDVFMKGLSK AKEGVVAAAE KTKQGVAAEA GKTKEGVLYV
GSKTKEGVVH GVATVAEKT EQVTNVGGAV VTGVTAVAQK
15 TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP
DNEAYEMPSE EGYQDYEPEA

[00042] The present invention relates to antibodies and to fragments of antibodies that are capable of immunospecifically binding to alpha-synuclein, and in particular to human alpha-synuclein, and in one embodiment exhibit the ability to immunospecifically bind to an epitope within amino acids 110-140 of human alpha-synuclein. According to some embodiments, the antibodies bind to an epitope within amino acids 112-117, 112-115, 118-126, 126-138 or 136-140 of human alpha-synuclein.
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[00043] By the term “taupathy” is typically referred to as neurodegenerative diseases associated with the pathological aggregation of tau. Typically, the taupathy is selected from the group consisting of Alzheimer’s disease, Argrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), Picks Disease, Primary age-related tauopathy
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(PART), Neurofibrillary tangle-predominant senile dementia, Dementia pugilistica, Chronic traumatic encephalopathy, stroke, stroke recovery, neurodegeneration in relation to Parkinson's disease, Parkinsonism linked to chromosome, LYTIC-BODIG disease (Parkinson-dementia complex of Guam), Ganglioglioma and gangliocytoma, Meningioangiomas, Postencephalitic parkinsonism, Subacute sclerosing panencephalitis, Huntington's disease, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease and lipofuscinosis. More typically, the tauopathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), and Picks Disease. In particular, the tauopathies may be selected from Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis due to AD or Psychosis in patients with AD, and psychiatric symptoms of patients with Lewy body dementia.

[00044] The term "antibody" (Ab) in the context of the present invention refers to an immunoglobulin molecule or according to some embodiments of the invention a fragment of an immunoglobulin molecule which has the ability to specifically bind to an epitope of a molecule ("antigen"). Naturally occurring antibodies typically comprise a tetramer which is usually composed of at least two heavy (H) chains and at least two light (L) chains. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region, usually comprised of three domains (CH1, CH2 and CH3). Heavy chains can be of any isotype, including IgG (IgG1, IgG2, IgG3 and IgG4). Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region (CL). Light chains include kappa chains and lambda chains. The heavy and light chain variable region is typically responsible for antigen recognition, while the heavy and light chain constant region may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (C1q) of the classical complement

system. The VH and VL regions can be further subdivided into regions of hypervariability, termed “complementarity determining regions,” that are interspersed with regions of more conserved sequence, termed “framework regions” (FR). Each VH and VL is composed of three CDR Domains and four FR Domains arranged from amino-terminus to carboxy-terminus in the following order: FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. Of particular relevance are antibodies and their epitope-binding fragments that have been “isolated” so as to exist in a physical milieu distinct from that in which it may occur in nature or that have been modified so as to differ from a naturally occurring antibody in amino acid sequence.

[00045] As used herein, the term “epitope-binding fragment of an antibody” means a fragment of an antibody capable of immunospecifically binding to an epitope. An epitope-binding fragment may contain 1, 2, 3, 4, 5 or all 6 of the CDR Domains of such antibody and, although capable of immunospecifically binding to such epitope, may exhibit an immunospecificity, affinity or selectivity toward such epitope that differs from that of such antibody. Preferably, however, an epitope-binding fragment will contain all 6 of the CDR Domains of such antibody. An epitope-binding fragment of an antibody may be a single polypeptide chain (*e.g.*, an scFv), or may comprise two or more polypeptide chains, each having an amino-terminus and a carboxyl terminus (*e.g.*, a diabody, an Fab fragment, an Fab₂ fragment, *etc.*). Fragments of antibodies that exhibit epitope-binding ability can be obtained, for example, by protease cleavage of intact antibodies. More preferably, although the two domains of the Fv fragment, VL and VH, are encoded by separate genes, such gene sequences or their encoding cDNA can be joined, using recombinant methods, by a flexible linker that enables them to be made as a single protein chain in which the VL and VH regions associate to form monovalent epitope-binding molecules (known as single-chain Fv (scFv); see *e.g.*, Bird *et al.*, (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. (U.S.A.)* 85:5879-5883). Alternatively, by employing a flexible linker that is too short (*e.g.*, less than about 9 residues) to enable the VL and VH regions of a single polypeptide chain to associate together, one can form a bispecific antibody,

diabody, or similar molecule (in which two such polypeptide chains associate together to form a bivalent epitope-binding molecule) (see for instance PNAS USA 90(14), 6444-8 (1993) for a description of diabodies). Examples of the epitope-binding fragments encompassed within the present invention include

5 (i) a Fab' or Fab fragment, a monovalent fragment consisting of the VL, VN, CL and CH1 domains, or a monovalent antibody as described in WO2007059782; (ii) F(ab')₂ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the VH and CH1 domains; (iv) a Fv fragment

10 consisting essentially of a VL and VH domains, (v) a dAb fragment (Ward et al., Nature 341, 544-546 (1989)), which consists essentially of a VH domain and also called domain antibodies (Holt et al; Trends Biotechnol. 2003 Nov;21(11) :484-90); (vi) camelid or nanobodies (Revets et al; Expert Opin Biol Ther. 2005 Jan;5_(I) : 11-24) and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment,

15 VL and VH, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see

20 for instance Bird et al., Science 242, 423-426 (1988) and Huston et al., PNAS USA 85, 5879-5883 (1988)). These and other useful antibody fragments in the context of the present invention are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes antibody-like polypeptides, such as chimeric antibodies and human-

25 ized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype. As used herein, "isotype" refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3,

30 IgG4) that is encoded by heavy chain constant region genes. Such antibody fragments are obtained using conventional techniques known to those of skill in the art; suitable fragments capable of binding to a desired epitope may be readily screened for utility in the same manner as an intact antibody.

- [00046]** The antibody GM37, 37 or GM37wt (used interchangeable herein) is intended to mean an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in CDR1-3 SEQ ID Nos 1-3 and the Light Chain CDR1-3 as given in SEQ ID Nos 4-6.
- 5 **[00047]** The variants 1, 2 and 3 of GM37 differs from GM37 by difference(s) in the CDR 2 sequence in the heavy chain as given in:
GM37 Variant 1 Heavy chain CDR2 SEQ ID NO: 33
GM37 Variant 2 Heavy chain CDR2 SEQ ID NO: 34
GM37 Variant 3 Heavy chain CDR2 SEQ ID NO: 35
- 10 **[00048]** The antibody GM285 or IgG-6004-285(used interchangeable herein) is intended to mean an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in CDR1-3 SEQ ID Nos 20-22 and the Light Chain CDR1-3 as given in SEQ ID Nos 23-25.
- [00049]** The antibody GM63 or 63 (used interchangeable herein) is intended to
- 15 mean an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in CDR1-3 SEQ ID Nos 51-53 and the Light Chain CDR1-3 as given in SEQ ID Nos 54-56.
- [00050]** The antibody 9E4 is intended to mean an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in
- 20 CDR1-3 SEQ ID Nos 44-46 and the Light Chain CDR1-3 as given in SEQ ID Nos 47-49.
- [00051]** The antibody 2E6 or m2E6 is intended to mean an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in CDR1-3 SEQ ID Nos 62-64 and the Light Chain CDR1-3 as given in
- 25 SEQ ID Nos 65-67.
- [00052]** The variants of 2E6, ch2E6, 2E6-HLD1, 2 or 3, has differences in their Heavy Chain and Light Chain outside the CDR regions as compared to 2E6
- [00053]** ch2E6 comprises or consist of a Heavy Chain SEQ ID NO: 70 and comprises or consist of a Light Chain SEQ ID NO: 71.
- 30 **[00054]** 2E6-HLD-1 comprises or consist of a Heavy Chain SEQ ID NO: 72 and comprises or consist of a Light Chain SEQ ID NO: 73.
- [00055]** 2E6-HLD-2 comprises or consist of a Heavy Chain SEQ ID NO: 74 and

comprises or consist of a Light Chain SEQ ID NO: 75.

[00056] 2E6-HLD-2 comprises or consist of a Heavy Chain SEQ ID NO: 76 and comprises or consist of a Light Chain SEQ ID NO: 77.

5 **[00057]** The affinity matured forms of HLD1: 7A10, 5A1, 9D7, 9G11, 7C4, L3, 8D9, 9C12 or 6B6 has differences in their CDR regions as defined in the sequence listing and claims compared to 2E6.

[00058] The antibody "6B6" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 120 and Heavy Chain SEQ ID NO 121.

10 **[00059]** The antibody "5A1" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 104 and Heavy Chain SEQ ID NO 105.

[00060] The antibody "9D7" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 106 and Heavy Chain SEQ ID NO 107.

[00061] The antibody "9G11" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 108 and Heavy Chain SEQ ID NO 109.

15 **[00062]** The antibody "L3" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 112 and Heavy Chain SEQ ID NO 113.

[00063] The antibody "7A10" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 114 and Heavy Chain SEQ ID NO 115.

20 **[00064]** The antibody "8D9" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 116 and Heavy Chain SEQ ID NO 117.

[00065] The antibody "9C12" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 118 and Heavy Chain SEQ ID NO 119.

[00066] The antibody "7C4" is intended to mean an antibody consisting of the Light Chain SEQ ID NO 110 and Heavy Chain SEQ ID NO 111.

25 **[00067]** Unless otherwise specified herein, the numbering of amino acid residues in this region is according to IMGT, Sequences of Proteins of Immunological

Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991).

[00068] The above-mentioned antibodies can be used in in treating patients with Alzheimer's disease or patients with a taupahty such as Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), Picks Disease, Primary age-related tauopathy (PART), Neurofibrillary tangle-predominant senile dementia, Dementia pugilistica, Chronic traumatic encephalopathy, stroke, stroke recovery, neurodegeneration in relation to Parkinson's disease, Parkinsonism linked to chromosome, Lytico-Bodig disease (Parkinson-dementia complex of Guam), Ganglioglioma and gangliocytoma, Meningioangiomas, Postencephalitic parkinsonism, Subacute sclerosing panencephalitis, Huntington's disease, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease and lipofuscinosis. More typically, the taupathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), and Picks Disease.

[00069] An "anti-alpha-synuclein" antibody is an antibody which binds specifically to alpha-synuclein or an alpha-synuclein fragment.

[00070] The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or during gene rearrangement or by somatic mutation in vivo).

[00071] The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

5 **[00072]** The antibodies of the present invention, and their alpha-synuclein epitope-binding fragments will preferably be "humanized," particularly if employed for therapeutic purposes. The term "humanized" refer to a chimeric molecule, generally prepared using recombinant techniques, having an antigen binding site derived from an immunoglobulin from a non-human species
10 and a remaining immunoglobulin structure based upon the structure and /or sequence of a human immunoglobulin. The antigen-binding site may comprise either complete non-human antibody variable domains fused to human constant domains, or only the complementarity determining regions (CDRs) of such variable domains grafted to appropriate human framework regions of
15 human variable domains. The framework residues of such humanized molecules may be wild type (*e.g.*, fully human) or they may be modified to contain one or more amino acid substitutions not found in the human antibody whose sequence has served as the basis for humanization. Humanization lessens or eliminates the likelihood that a constant region of the molecule will act as
20 an immunogen in human individuals, but the possibility of an immune response to the foreign variable region remains (LoBuglio, A.F. *et al.* (1989) "Mouse/Human Chimeric Monoclonal Antibody In Man: Kinetics And Immune Response," Proc. Natl. Acad. Sci. (U.S.A.) 86:4220-4224). Another approach focuses not only on providing human-derived constant regions, but modifying
25 the variable regions as well so as to reshape them as closely as possible to human form. It is known that the variable regions of both heavy and light chains contain three complementarity- determining regions (CDRs) which vary in response to the antigens in question and determine binding capability, flanked by four framework regions (FRs) which are relatively conserved in a
30 given species and which putatively provide a scaffolding for the CDRs. When non-human antibodies are prepared with respect to a particular antigen, the variable regions can be "reshaped" or "humanized" by grafting CDRs derived from nonhuman antibody on the FRs present in the human antibody to be

modified. Application of this approach to various antibodies has been reported by Sato, K. *et al.* (1993) *Cancer Res* 53:851-856. Riechmann, L. *et al.* (1988) "*Reshaping Human Antibodies for Therapy*," *Nature* 332:323-327; Verhoeven, M. *et al.* (1988) "*Reshaping Human Antibodies: Grafting An Antilysozyme Activity*," *Science* 239:1534-1536; Kettleborough, C. A. *et al.* (1991) "Humanization Of A Mouse Monoclonal Antibody By CDR-Grafting: The Importance Of Framework Residues On Loop Conformation," *Protein Engineering* 4:773-3783; Maeda, H. *et al.* (1991) "*Construction Of Reshaped Human Antibodies With HIV-Neutralizing Activity*," *Human Antibodies Hybridoma* 2:124-134; Gorman, S. D. *et al.* (1991) "*Reshaping A Therapeutic CD4 Antibody*," *Proc. Natl. Acad. Sci. (U.S.A.)* 88:4181-4185; Tempest, P.R. *et al.* (1991) "*Reshaping A Human Monoclonal Antibody To Inhibit Human Respiratory Syncytial Virus Infection in vivo*," *Bio/Technology* 9:266-271; Co, M. S. *et al.* (1991) "*Humanized Antibodies For Antiviral Therapy*," *Proc. Natl. Acad. Sci. (U.S.A.)* 88:2869-2873; Carter, P. *et al.* (1992) "*Humanization Of An Anti-p185her2 Antibody For Human Cancer Therapy*," *Proc. Natl. Acad. Sci. (U.S.A.)* 89:4285-4289; and Co, M.S. *et al.* (1992) "*Chimeric And Humanized Antibodies With Specificity For The CD33 Antigen*," *J. Immunol.* 148:1149-1154. In some embodiments, humanized antibodies preserve all CDR sequences (for example, a humanized mouse antibody which contains all six CDRs from the mouse antibodies). In other embodiments, humanized antibodies have one or more CDRs (one, two, three, four, five, six) which are altered with respect to the original antibody, which are also termed one or more CDRs "derived from" one or more CDRs from the original antibody. The ability to humanize an antigen is well known (see, *e.g.*, US Patents No. 5,225,539; 5,530,101; 5,585,089; 5,859,205; 6,407,213; 6,881,557).

[00073] As used herein, an antibody or an epitope-binding antigen-binding fragment thereof is said to "immunospecifically" bind a region of another molecule (*i.e.*, an epitope) if it reacts or associates more frequently, more rapidly, with greater duration and/or with greater affinity or avidity with that epitope relative to alternative epitopes. It is also understood by reading this definition that, for example, an antibody or an epitope-binding antigen-binding fragment thereof

that specifically binds to a first target may or may not specifically or preferentially bind to a second target. As used herein, the term "binding" in the context of the binding of an antibody to a predetermined antigen typically refers to binding with an affinity corresponding to a KD of about 10^{-7} M or less, such as about 10^{-8} M or less, such as about 10^{-9} M or less when determined by for instance surface plasmon resonance (SPR) technology in a BIAcore 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a KD that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The amount with which the affinity is lower is dependent on the KD of the antibody, so that when the KD of the antibody is very low (that is, the antibody is highly specific), then the amount with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold.

[00074] The term " k_d " (sec^{-1} or $1/\text{s}$), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. Said value is also referred to as the k_{off} value.

[00075] The term " k_a " ($\text{M}^{-1} \times \text{sec}^{-1}$ or $1/\text{M}$), as used herein, refers to the association rate constant of a particular antibody-antigen interaction.

[00076] The term " KD " (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

[00077] The term " KA " (M^{-1} or $1/\text{M}$), as used herein, refers to the association equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the k_a by the k_d .

[00078] In some antibodies only part of a CDR, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (see, Kabat *et al.* (1992) SEQUENCES OF

PROTEINS OF IMMUNOLOGICAL INTEREST, National Institutes of Health Publication No. 91-3242; Chothia, C. *et al.* (1987) "*Canonical Structures For The Hypervariable Regions Of Immunoglobulins*," J. Mol. Biol. 196:901-917), by molecular modeling and/or empirically, or as described in Gonzales, N.R. *et al.* (2004) "*SDR Grafting Of A Murine Antibody Using Multiple Human Germline Templates To Minimize Its Immunogenicity*," Mol. Immunol. 41:863-872. In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

[00079] The fact that a single amino acid alteration of a CDR residue can result in loss of functional binding (Rudikoff, S. *et al.* (1982) "*Single Amino Acid Substitution Altering Antigen-Binding Specificity*," Proc. Natl. Acad. Sci. (USA) 79(6):1979-1983) provides a means for systematically identifying alternative functional CDR sequences. In one preferred method for obtaining such variant CDRs, a polynucleotide encoding the CDR is mutagenized (for example via random mutagenesis or by a site-directed method (*e.g.*, polymerase chain-mediated amplification with primers that encode the mutated locus)) to produce a CDR having a substituted amino acid residue. By comparing the identity of the relevant residue in the original (functional) CDR sequence to the identity of the substituted (non-functional) variant CDR sequence, the BLOSUM62.ijj substitution score for that substitution can be identified. The BLOSUM system provides a matrix of amino acid substitutions created by analyzing a database of sequences for trusted alignments (Eddy, S.R. (2004) "*Where Did The BLOSUM62 Alignment Score Matrix Come From?*," Nature

Biotech. 22(8):1035-1036; Henikoff, J.G. (1992) "Amino acid substitution matrices from protein blocks," Proc. Natl. Acad. Sci. (USA) 89:10915-10919; Karlin, S. *et al.* (1990) "*Methods For Assessing The Statistical Significance Of Molecular Sequence Features By Using General Scoring Schemes*," Proc. Natl. Acad. Sci. (USA) 87:2264-2268; Altschul, S.F. (1991) "*Amino Acid Substitution Matrices From An Information Theoretic Perspective*," J. Mol. Biol. 219, 555-565. Currently, the most advanced BLOSUM database is the BLOSUM62 database (BLOSUM62.iij). **Table 1** presents the BLOSUM62.iij substitution scores (the higher the score the more conservative the substitution and thus the more likely the substitution will not affect function). If an antigen-binding fragment comprising the resultant CDR fails to bind to ROR1, for example, then the BLOSUM62.iij substitution score is deemed to be insufficiently conservative, and a new candidate substitution is selected and produced having a higher substitution score. Thus, for example, if the original residue was glutamate (E), and the non-functional substitute residue was histidine (H), then the BLOSUM62.iij substitution score will be 0, and more conservative changes (such as to aspartate, asparagine, glutamine, or lysine) are preferred.

Table 1																				
	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	+4	-1	-2	-2	0	-1	-1	0	-2	-1	-1	-1	-1	-2	-1	+1	0	-3	-2	0
R	-1	+5	0	-2	-3	+1	0	-2	0	-3	-2	+2	-1	-3	-2	-1	-1	-3	-2	-3
N	-2	0	+6	+1	-3	0	0	0	+1	-3	-3	0	-2	-3	-2	+1	0	-4	-2	-3
D	-2	-2	+1	+6	-3	0	+2	-1	-1	-3	-4	-1	-3	-3	-1	0	-1	-4	-3	-3
C	0	-3	-3	-3	+9	-3	-4	-3	-3	-1	-1	-3	-1	-2	-3	-1	-1	-2	-2	-1
Q	-1	+1	0	0	-3	+5	+2	-2	0	-3	-2	+1	0	-3	-1	0	-1	-2	-1	-2
E	-1	0	0	+2	-4	+2	+5	-2	0	-3	-3	+1	-2	-3	-1	0	-1	-3	-2	-2
G	0	-2	0	-1	-3	-2	-2	+6	-2	-4	-4	-2	-3	-3	-2	0	-2	-2	-3	-3
H	-2	0	+1	-1	-3	0	0	-2	+8	-3	-3	-1	-2	-1	-2	-1	-2	-2	+2	-3
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	+4	+2	-3	+1	0	-3	-2	-1	-3	-1	+3
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	+2	+4	-2	+2	0	-3	-2	-1	-2	-1	+1
K	-1	+2	0	-1	-3	+1	+1	-2	-1	-3	-2	+5	-1	-3	-1	0	-1	-3	-2	-2
M	-1	-1	-2	-3	-1	0	-2	-3	-2	+1	+2	-1	+5	0	-2	-1	-1	-1	-1	+1
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	+6	-4	-2	-2	+1	+3	-1
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	+7	-1	-1	-4	-3	-2
S	+1	-1	+1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	+4	+1	-3	-2	-2
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	+1	+5	-2	-2	0
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	+1	-4	-3	-2	+11	+2	-3
Y	-2	-2	-2	-3	-2	-1	-2	-3	+2	-1	-1	-2	-1	+3	-3	-2	-2	+2	+7	-1
V	0	-3	-3	-3	-1	-2	-2	-3	-3	+3	+1	-2	+1	-1	-2	-2	0	-3	-1	+4

[00080] The invention thus contemplates the use of random mutagenesis to identify improved CDRs. In the context of the present invention, conservative substitutions may be defined by substitutions within the classes of amino acids reflected in one or more of the following three tables:

5

Amino Acid Residue Classes For Conservative Substitutions:

Table 2	
Acidic Residues	Asp (D) and Glu (E)
Basic Residues	Lys (K), Arg (R), and His (H)
Hydrophilic Uncharged Residues	Ser (S), Thr (T), Asn (N), and Gln (Q)

Aliphatic Uncharged Residues	Cly (G), Ala (A), Val (V), Leu (L), and Ile (I)
Non-polar Uncharged Residues	Cys (C), Met (M), and Pro (P)
Aromatic Residues	Phe (F), Tyr (Y), and Trp (W)

Alternative Conservative Amino Acid Residue Substitution Classes:

Table 3			
1	A	S	T
2	D	E	
3	N	Q	
4	R	K	
5	I	L	M
6	F	Y	W

Alternative Physical and Functional Classifications of Amino Acid Residues:

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Table 4	
Alcohol Group-Containing Residues	S and T
Aliphatic Residues	I, L, V and M
Cycloalkenyl-Associated Residues	F, H, W and Y
Hydrophobic Residues	A, C, F, G, H, I, L, M, R, T, V, W and Y
Negatively Charged Residues	D and E
Polar Residues	C, D, E, H, K, N, Q, R, S and T
Positively Charged Residues	H, K and R
Small Residues	A, C, D, G, N, P, S, T and V
Very Small Residues	A, G and S
Residues Involved In Turn Formation	A, C, D, E, G, H, K, N, Q, R, S, P and T
Flexible Residues	Q, T, K, S, G, P, D, E and R

[00081] More conservative substitutions groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

5 [00082] Additional groups of amino acids may also be formulated using the principles described in, e.g., Creighton (1984) *Proteins: Structure and Molecular Properties* (2d Ed. 1993), W. H. Freeman and Company.

10 [00083] Phage display technology can alternatively be used to increase (or decrease) CDR affinity. This technology, referred to as affinity maturation, employs mutagenesis or "CDR walking" and re-selection uses the target antigen or an antigenic antigen-binding fragment thereof to identify antibodies having CDRs that bind with higher (or lower) affinity to the antigen when compared with the initial or parental antibody (See, e.g. Glaser *et al.* (1992) *J. Immunology* 149:3903). Mutagenizing entire codons rather than single nucleotides results in a semi-randomized repertoire of amino acid mutations. Libraries can be constructed consisting of a pool of variant clones each of which differs by a single amino acid alteration in a single CDR and which contain variants representing each possible amino acid substitution for each CDR residue. Mutants with increased (or decreased) binding affinity for the antigen can be screened by contacting the immobilized mutants with labelled antigen. Any screening method known in the art can be used to identify mutant antibodies with increased or decreased affinity to the antigen (e.g., ELISA) (See Wu *et al.* 1998, *Proc. Natl. Acad. Sci. (U.S.A.)* 95:6037; Yelton *et al.*, 1995, *J. Immunology* 155:1994). CDR walking which randomizes the Light Chain may be used possible (see, Schier *et al.*, 1996, *J. Mol. Bio.* 263:551).

25 [00084] Methods for accomplishing such affinity maturation are described for example in: Krause, J.C. *et al.* (2011) "An Insertion Mutation That Distorts Antibody Binding Site Architecture Enhances Function Of A Human Antibody," *MBio.* 2(1) pii: e00345-10. doi: 10.1128/mBio.00345-10; Kuan, C.T. *et al.* (2010) "Affinity-Matured Anti-Glycoprotein NMB Recombinant Immunotoxins Targeting Malignant Gliomas And Melanomas," *Int. J. Cancer* 10.1002/ijc.25645; Hackel, B.J. *et al.* (2010) "Stability And CDR Composition Biases Enrich Binder Functionality Landscapes," *J. Mol. Biol.* 401(1):84-96; Montgomery, D.L. *et al.* (2009) "Affinity Maturation And Characterization Of A

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 man Antibody Library And Directed Against The Internal Trimeric Coiled-Coil
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[00085] Thus, the sequence of CDR variants of encompassed antibodies or their
 epitope-binding fragments may differ from the sequence of the CDR of the
 parent antibody through substitutions; for instance substituted 4 amino acid
 residue, 3 amino acid residue, 2 amino acid residue or 1 of the amino acid
 20 residues. According to an embodiment of the invention it is furthermore envis-
 aged that the amino acids in the CDR regions may be substituted with con-
 servative substitutions, as defined in the below 3 tables. For example, the
 acidic residue Asp can be substituted with Glu without substantially affecting
 the binding characteristic of the antibody.

[00086] The term "epitope" means an antigenic determinant capable of immuno-
 specific binding to an antibody. Epitopes usually consist of surface groupings
 of molecules such as amino acids or sugar side chains and usually have spe-
 cific three dimensional structural characteristics, as well as specific charge
 characteristics. Conformational and nonconformational epitopes are distin-
 25 guished in that the binding to the former, but not the latter, is lost in the pres-
 ence of denaturing solvents. The epitope may comprise amino acid residues
 directly involved in the binding (also called immunodominant component of
 the epitope) and other amino acid residues, which are not directly involved in
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the binding, such as amino acid residues which are effectively blocked by the specifically antigen binding peptide (in other words, the amino acid residue is within the footprint of the specifically antigen binding peptide).

[00087] The term "transgenic non-human animal" refers to a non-human animal
5 having a genome comprising one or more human heavy and/or light chain
transgenes or transchromosomes (either integrated or non-integrated into the
animal's natural genomic DNA) and which is capable of expressing fully hu-
man antibodies. For example, a transgenic mouse can have a human light
chain transgene and either a human heavy chain transgene or human heavy
10 chain transchromosome, such that the mouse produces human anti-alpha-
synuclein antibody when immunized with alpha-synuclein antigen and/or cells
expressing alpha-synuclein. The human heavy chain transgene may be inte-
grated into the chromosomal DNA of the mouse, as is the case for transgenic
mice, for instance HuMAb mice, such as HCo7 or HCo2 mice, or the human
15 heavy chain transgene may be maintained extrachromosomally, as is the
case for transchromosomal KM mice as described in WO02/43478. Such
transgenic and transchromosomal mice (collectively referred to herein as
"transgenic mice") are capable of producing multiple isotypes of human mon-
oclonal antibodies to a given antigen (such as IgG, IgA, IgM, IgD and/or IgE)
20 by undergoing V-D-J recombination and isotype switching.

[00088] Transgenic, nonhuman animal can also be used for production of anti-
bodies against a specific antigen by introducing genes encoding such specific
antibody, for example by operatively linking the genes to a gene which is ex-
pressed in the milk of the animal.

25 **[00089]** The term "treatment" or "treating" as used herein means ameliorating,
slowing or reversing the progress or severity of a disease or disorder, or ame-
liorating, slowing or reversing one or more symptoms or side effects of such
disease or disorder. For purposes of this invention, "treatment" or "treating"
further means an approach for obtaining beneficial or desired clinical results,
30 where "beneficial or desired clinical results" include, without limitation, allevia-
tion of a symptom, diminishment of the extent of a disorder or disease, stabi-
lized (i.e., not worsening) disease or disorder state, delay or slowing of the
progression a disease or disorder state, amelioration or palliation of a disease

or disorder state, and remission of a disease or disorder, whether partial or total, detectable or undetectable.

5 [00090] An "effective amount," when applied to an antibody of the invention, refers to an amount sufficient, at dosages and for periods of time necessary, to achieve an intended biological effect or a desired therapeutic result including, without limitation, clinical results. The phrase "therapeutically effective amount" when applied to an antibody of the invention is intended to denote an amount of the antibody that is sufficient to ameliorate, palliate, stabilize, reverse, slow or delay the progression of a disorder or disease state, or of a
10 symptom of the disorder or disease. In an embodiment, the method of the present invention provides for administration of the antibody in combinations with other compounds. In such instances, the "effective amount" is the amount of the combination sufficient to cause the intended biological effect.

15 [00091] A therapeutically effective amount of an anti-alpha-synuclein antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the anti-alpha-synuclein antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

20 [00092] As indicated above, the present invention particularly relates to a monoclonal antibody capable of immunospecifically binding to an epitope within amino acids 110-140 of human alpha-synuclein. In one embodiment the antibody is capable of competing with the antibody GM37 for binding the 112-117 epitope of alpha-synuclein. In another embodiment the antibody is capable of competing with the antibody GM285 for binding the 112-115 epitope of alpha-synuclein, In another embodiment the antibody is capable of competing with the antibody GM63 for binding the 126-138 epitope of alpha-synuclein. In another embodiment the antibody is capable of competing with the antibody 2E6 for binding the 126-140 epitope of alpha-synuclein. In yet another embodiment the antibody is capable of competing with the antibody 9E4 for binding the 118-126 epitope of alpha-synuclein.
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[00093] The antibody is preferably a human or humanized antibody.

[00094] The antibodies of the invention is further defined in the claims

[00095] The present invention also provides a method of reducing Tau tangle formation in a patient, comprising administering to the patient in need of such treatment, a therapeutically effective amount of an antibody of the invention.

[00096] Further the antibodies may be in a composition together with a pharmaceutically acceptable carrier, diluent and/or stabilizer. The antibodies of the invention may be used in therapy. In particular the antibodies of the invention may be used in treating a tauopathy. Typically, the tauopathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), Picks Disease, Primary age-related tauopathy (PART), Neurofibrillary tangle-predominant senile dementia, Dementia pugilistica, Chronic traumatic encephalopathy, stroke, stroke recovery, neurodegeneration in relation to Parkinson's disease, Parkinsonism linked to chromosome, Lytico-Bodig disease (Parkinson-dementia complex of Guam), Ganglioglioma and gangliocytoma, Meningioangiomas, Postencephalitic parkinsonism, Subacute sclerosing panencephalitis, Huntington's disease, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease and lipofuscinosis. More typically, the tauopathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), and Picks Disease. In particular, the tauopathies may be selected from Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis due to AD or Psychosis in patients with AD, and psychiatric symptoms of patients with Lewy body dementia.

[00097] The treatment may be chronic and the patient may be treated at least 2 weeks, such as at least for 1 month, 6, months, 1 year or more.

[00098] The antibodies of the present invention may for example be monoclonal antibodies produced by the hybridoma method first described by Kohler et al.,

Nature 256, 495 (1975), or may be produced by recombinant DNA methods. Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in, for example, Clackson et al., Nature 352, 624-628 (1991) and Marks et al., J. Mol. Biol. 222, 581-597 (1991). Monoclonal antibodies may be obtained from any suitable source. Thus, for example, monoclonal antibodies may be obtained from hybridomas prepared from murine splenic B lymphocyte cells obtained from mice immunized with an antigen of interest, for instance, in the form of cells expressing the antigen on the surface, or a nucleic acid encoding an antigen of interest. Monoclonal antibodies may also be obtained from hybridomas derived from antibody-expressing cells of immunized humans or from non-human mammals such as rats, rabbits, dogs, sheep, goats, primates, etc.

[00099] In one embodiment, the antibody of the invention is a human antibody. Human monoclonal antibodies directed against alpha-synuclein may be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. Such transgenic and transchromosomal mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "transgenic mice".

[000100] The HuMAb mouse contains a human immunoglobulin gene minilocus that encodes unrearranged human heavy variable and constant (μ and Y) and light variable and constant (K) chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and K chain loci (Lonberg, N. et al., Nature 368, 856-859 (1994)). Accordingly, such mice exhibit reduced expression of mouse IgM or IgK and in response to immunization, the introduced human heavy and light chain transgenes, undergo class switching and somatic mutation to generate high affinity human IgG, κ monoclonal antibodies (Lonberg, N. et al. (1994), supra; reviewed in Lonberg, N., Handbook of Experimental Pharmacology 113, 49-101 (1994) , Lonberg, N. and Huszar, D., Intern. Rev. Immunol. Vol. 13 65-93 (1995) and Harding, F. and Lonberg, N., Ann. N. Y. Acad. Sci 764 536-546 (1995)). The preparation of HuMAb mice is described in detail in Taylor, L. et al., Nucleic Acids Research 20, 6287-6295 (1992), Chen, J. et al., International Immunology 5,

647-656 (1993), Tuaillon et al., J. Immunol. 152, 2912-2920 (1994), Taylor, L. et al., International Immunology 6, 579-591 (1994), Fishwild, D. et al., Nature Biotechnology 14, 845-851 (1996). See also US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,789,650, US 5,877,397, US 5,661,016, US 5,814,318, US 5,874,299, US 5,770,429, US 5,545,807, WO 98/24884, WO 94/25585, WO 93/1227, WO 92/22645, WO 92/03918 and WO 01/09187.

[000101] The HCo7 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen et al., EMBO J. 12, 821-830 (1993)), a CMD disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), a KCo5 human kappa light chain transgene (as described in Fishwild et al., Nature Biotechnology 14, 845-851 (1996)), and a HCo7 human heavy chain transgene (as described in US 5,770,429).

[000102] The HCo2 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen et al., EMBO J. 12, 821-830 (1993)), a CMD disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), a KCo5 human kappa light chain transgene (as described in Fishwild et al., Nature Biotechnology 14, 845-851 (1996)), and a HCo2 human heavy chain transgene (as described in Example 2 of WO 01/14424).

[000103] In the KM mouse strain, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen et al., EMBO J. 12, 811-820 (1993) and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of WO 01/09187. This mouse strain carries a human kappa light chain transgene, KCo5, as described in Fishwild et al., Nature Biotechnology 14, 845-851 (1996). This mouse strain also carries a human heavy chain transchromosome composed of chromosome 14 fragment hCF (SC20) as described in WO 02/43478.

[000104] Splenocytes from these transgenic mice may be used to generate hybridomas that secrete human monoclonal antibodies according to well-known techniques. Human monoclonal of the present invention, or antibodies of the present invention originating from other species may also be generated

transgenically through the generation of another non-human mammal or plant that is transgenic for the immunoglobulin heavy and light chain sequences of interest and production of the antibody in a recoverable form therefrom. In connection with the transgenic production in mammals, antibodies may be produced in, and recovered from, the milk of goats, cows, or other mammals. See for instance US 5,827,690, US 5,756,687, US 5,750,172 and US 5,741,957.

[000105] The antibody of the invention may be of any isotype. The choice of isotype typically will be guided by the desired effector functions, such as ADCC induction. Exemplary isotypes are IgG1, IgG2, IgG3, and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. If desired, the class of an anti-alpha-synuclein antibody of the present invention may be switched by known methods. For example, an antibody of the present invention that was originally IgM may be class switched to an IgG antibody of the present invention. Further, class switching techniques may be used to convert one IgG subclass to another, for instance from IgG1 to IgG2. Thus, the effector function of the antibodies of the present invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses. In one embodiment an antibody of the present invention is an IgG1 antibody, for instance an IgG1, κ .

[000106] In one embodiment, the antibody of the invention is a full-length antibody, preferably an IgG antibody, in particular an IgG1, κ antibody. In another embodiment, the antibody of the invention is an antibody fragment or a single-chain antibody.

[000107] Antibodies fragments may e.g. be obtained by fragmentation using conventional techniques, and the fragments screened for utility in the same manner as described herein for whole antibodies. For example, F(ab')₂ fragments may be generated by treating antibody with pepsin. The resulting F(ab')₂ fragment may be treated to reduce disulfide bridges to produce Fab' fragments. Fab fragments may be obtained by treating an IgG antibody with papain; Fab' fragments may be obtained with pepsin digestion of IgG antibody. An F(ab') fragment may also be produced by binding Fab' described below via a thioether bond or a disulfide bond. A Fab' fragment is an antibody

fragment obtained by cutting a disulfide bond of the hinge region of the F(ab')₂. A Fab' fragment may be obtained by treating an F(ab')₂ fragment with a reducing agent, such as dithiothreitol. Antibody fragment may also be generated by expression of nucleic acids encoding such fragments in recombinant cells (see for instance Evans et al., J. Immunol. Meth. 184, 123-38 (1995)). For example, a chimeric gene encoding a portion of an F(ab')₂ fragment could include DNA sequences encoding the CH1 domain and hinge region of the H chain, followed by a translational stop codon to yield such a truncated antibody fragment molecule.

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- 10 **[000108]** In one embodiment, the anti-alpha-synuclein antibody is a monovalent antibody, preferably a monovalent antibody as described in WO2007059782 (which is incorporated herein by reference in its entirety) having a deletion of the hinge region. Accordingly, in one embodiment, the antibody is a monovalent antibody, wherein said anti-alpha-synuclein antibody is constructed by a method comprising: i) providing a nucleic acid construct encoding the light chain of said monovalent antibody, said construct comprising a nucleotide sequence encoding the VL region of a selected antigen specific anti-alpha-synuclein antibody and a nucleotide sequence encoding the constant CL region of an Ig, wherein said nucleotide sequence encoding the VL region of a selected antigen specific antibody and said nucleotide sequence encoding the CL region of an Ig are operably linked together, and wherein, in case of an IgG1 subtype, the nucleotide sequence encoding the CL region has been modified such that the CL region does not contain any amino acids capable of forming disulfide bonds or covalent bonds with other peptides comprising an identical amino acid sequence of the CL region in the presence of polyclonal human IgG or when administered to an animal or human being; ii) providing a nucleic acid construct encoding the heavy chain of said monovalent antibody, said construct comprising a nucleotide sequence encoding the VH region of a selected antigen specific antibody and a nucleotide sequence encoding a constant CH region of a human Ig, wherein the nucleotide sequence encoding the CH region has been modified such that the region corresponding to the hinge region and, as required by the Ig subtype, other regions of the CH region, such as the CH3 region, does not comprise
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any amino acid residues which participate in the formation of disulphide bonds or covalent or stable non-covalent inter-heavy chain bonds with other peptides comprising an identical amino acid sequence of the CH region of the human Ig in the presence of polyclonal human IgG or when administered to an animal human being, wherein said nucleotide sequence encoding the VH region of a selected antigen specific antibody and said nucleotide sequence encoding the CH region of said Ig are operably linked together; iii) providing a cell expression system for producing said monovalent antibody; iv) producing said monovalent antibody by co-expressing the nucleic acid constructs of (i) and (ii) in cells of the cell expression system of (iii).

[000109] Similarly, in one embodiment, the anti- alpha-synuclein antibody is a monovalent antibody, which comprises:

- (i) a variable region of an antibody of the invention as described herein or an antigen binding part of the said region, and
- (ii) a CH region of an immunoglobulin or a antigen-binding fragment thereof comprising the CH2 and CH3 regions, wherein the CH region or antigen-binding fragment thereof has been modified such that the region corresponding to the hinge region and, if the immunoglobulin is not an IgG4 subtype, other regions of the CH region, such as the CH3 region, do not comprise any amino acid residues, which are capable of forming disulfide bonds with an identical CH region or other covalent or stable non-covalent inter-heavy chain bonds with an identical CH region in the presence of polyclonal human IgG.

[000110] In a further embodiment, the heavy chain of the monovalent anti-alpha-synuclein antibody has been modified such that the entire hinge has been deleted.

[000111] In another further embodiment, the sequence of said monovalent antibody has been modified so that it does not comprise any acceptor sites for N-linked glycosylation.

[000112] Anti-alpha-synuclein antibodies of the invention also include single chain antibodies. Single chain antibodies are peptides in which the heavy and light chain Fv regions are connected. In one embodiment, the present invention provides a single-chain Fv (scFv) wherein the heavy and light chains in the Fv of an anti-alpha-synuclein antibody of the present invention are joined

with a flexible peptide linker (typically of about 10, 12, 15 or more amino acid residues) in a single peptide chain. Methods of producing such antibodies are described in for instance US 4,946,778, Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994), Bird et al., *Science* 242, 423-426 (1988), Huston et al., *PNAS USA* 85, 5879-5883 (1988) and McCafferty et al., *Nature* 348, 552-554 (1990). The single chain antibody may be monovalent, if only a single VH and VL are used, bivalent, if two VH and VL are used, or polyvalent, if more than two VH and VL are used.

10 **[000113]** In general, anti-alpha-synuclein antibodies described herein may be modified by inclusion of any suitable number of modified amino acids and/or associations with such conjugated substituents. Suitability in this context is generally determined by the ability to at least substantially retain alpha-synuclein selectivity and/or the anti-alpha-synuclein specificity associated
15 with the non-derivatized parent anti-alpha-synuclein antibody. The inclusion of one or more modified amino acids may be advantageous in, for example, increasing polypeptide serum half-life, reducing polypeptide antigenicity, or increasing polypeptide storage stability. Amino acid(s) are modified, for example, co-translationally or post-translationally during recombinant production
20 (e.g., N-linked glycosylation at N-X-S/T motifs during expression in mammalian cells) or modified by synthetic means. Non-limiting examples of a modified amino acid include a glycosylated amino acid, a sulfated amino acid, a prenylated (e. g., farnesylated, geranylgeranylated) amino acid, an acetylated amino acid, an acylated amino acid, a PEGylated amino acid, a biotinylated
25 amino acid, a carboxylated amino acid, a phosphorylated amino acid, and the like. References adequate to guide one of skill in the modification of amino acids are replete throughout the literature. Example protocols are found in Walker (1998) *Protein Protocols On CD-Rom*, Humana Press, Totowa, NJ. The modified amino acid may, for instance, be selected from a glycosylated
30 amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, or an amino acid conjugated to an organic derivatizing agent.

[000114] Anti-alpha-synuclein antibodies may also be chemically modified by covalent conjugation to a polymer to for instance increase their circulating half-life. Exemplary polymers, and methods to attach them to peptides, are illustrated in for instance US 4,766,106, US 4,179,337, US 4,495,285 and US 4,609,546. Additional illustrative polymers include polyoxyethylated polyols and polyethylene glycol (PEG) (e.g., a PEG with a molecular weight of between about 1,000 and about 40,000, such as between about 2,000 and about 20,000, e.g., about 3,000-12,000 g/mol).

[000115] In a aspect, the invention relates to a pharmaceutical composition comprising:

- an anti-alpha-synuclein antibody as defined herein, and
- a pharmaceutically-acceptable carrier.

[000116] The pharmaceutical compositions may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in Remington : The Science and Practice of Pharmacy, 21th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2005.

[000117] The pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients should be suitable for the chosen compound of the present invention and the chosen mode of administration. Suitability for carriers and other components of pharmaceutical compositions is determined based on the lack of significant negative impact on the desired biological properties of the chosen compound or pharmaceutical composition of the present invention (e.g., less than a substantial impact (10% or less relative inhibition, 5% or less relative inhibition, etc.)) on antigen binding.

[000118] A pharmaceutical composition of the present invention may also include diluents, fillers, salts, buffers, detergents (e.g., a nonionic detergent, such as Tween-20 or Tween- 80), stabilizers (e.g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition. The diluent is selected to not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution. In addition, the

pharmaceutical composition or formulation may also include other carriers, or non-toxic, nontherapeutic, non-immunogenic stabilizers and the like. The compositions may also include large, slowly metabolized macromolecules, such as proteins, polysaccharides like chitosan, polylactic acids, polyglycolic acids and copolymers (e.g., latex functionalized sepharose, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (e.g., oil droplets or liposomes).

[000119] The actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

[000120] The pharmaceutical composition may be administered by any suitable route and mode, including: parenteral, topical, oral or intranasal means for prophylactic and/or therapeutic treatment. In one embodiment, a pharmaceutical composition of the present invention is administered parenterally. The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

[000121] Additional suitable routes of administering a compound of the present invention in vivo and in vitro are well known in the art and may be selected by those of ordinary skill in the art.

[000122] In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

[000123] Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption delaying agents, and the like that are physiologically compatible with a compound of the present invention.

[000124] Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, corn oil, peanut oil, cottonseed oil, and sesame oil, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Other carriers are well known in the pharmaceutical arts.

[000125] Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated.

[000126] Pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

[000127] Pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

[000128] The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The compounds of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

[000129] In one embodiment, the compounds of the present invention may be formulated to ensure proper distribution in vivo. Pharmaceutically acceptable carriers for parenteral administration include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. The use of such media and agents for pharmaceutically active substances is known in the art. Except insofar as any conventional media or agent is incompatible with the active compound, use thereof in the pharmaceutical compositions of the present invention is contemplated. Supplementary active compounds may also be incorporated into the compositions.

[000130] Pharmaceutical compositions for injection must typically be sterile and stable under the conditions of manufacture and storage. The composition may be formulated as a solution, microemulsion, liposome, or other ordered structure suitable to high drug concentration. The carrier may be a aqueous or nonaqueous solvent or dispersion medium containing for instance water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. The proper fluidity may 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. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as glycerol, mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions may be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000131] Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

[000132] Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of

administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the subjects to be treated; each unit contains a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the present invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

10 **[000133]** The effective dosages and the dosage regimens for the anti alpha-synuclein antibodies depend on the disease or condition to be treated and may be determined by the persons skilled in the art. On any given day that a dosage is given, the dosage may range from about 0.0001 to about 100 mg/kg, and more usually from about 0.01 to about 5 mg/kg, of the host body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg body weight. Exemplary dosages thus include: from about 0.1 to about 10 mg/kg/body weight, from about 0.1 to about 5 mg/kg/body weight, from about 0.1 to about 2 mg/kg/body weight, from about 0.1 to about 1 mg/kg/body weight, for instance about 0.15 mg/kg/body weight, about 0.2 mg/kg/body weight, about 0.5 mg/kg/body weight, about 1 mg/kg/body weight, about 1.5 mg/kg/body weight, about 2 mg/kg/body weight, about 5 mg/kg/body weight, or about 10 mg/kg/body weight.

25 **[000134]** A physician or veterinarian having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the anti-alpha-synuclein antibody employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Such an effective dose will generally de-

pend upon the factors described above. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous, and for instance administered proximal to the site of the target. If desired, the effective daily dose of a pharmaceutical composition may be administered as two, three, four,
5 five, six or more sub-doses administered separately at appropriate intervals throughout the day, optionally, in unit dosage forms. While it is possible for a compound of the present invention to be administered alone, it is preferable to administer the compound as a pharmaceutical composition as described above.

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Sequence Listing

SEQ ID NO:1	GM37 CDR 1 Heavy Chain
SEQ ID NO:2	GM37 CDR 2 Heavy Chain
SEQ ID NO:3	GM37 CDR 3 Heavy Chain
SEQ ID NO:4	GM37 CDR 1 Light Chain
SEQ ID NO:5	GM37 CDR 2 Light Chain
SEQ ID NO:6	GM37 CDR 3 Light Chain
SEQ ID NO:7	GM37 Heavy Chain Variable Domain
SEQ ID NO:8	GM37 Light Chain Variable Domain
SEQ ID NO:9	Epitope 112-117 of Human Alpha-Synuclein
SEQ ID NO:10	Human Alpha-Synuclein
SEQ ID NO:11	A-Syn-AAKK-BAP
SEQ ID NO:12	A-Syn-BAAK-BAP
SEQ ID NO:13	A-Syn-BBAA-BAP
SEQ ID NO:14	A-Syn-BBKK-BAP
SEQ ID NO:15	A-Syn-120-140_Del-BAP
SEQ ID NO:16	Residues 1-119 of Human Alpha-Synuclein
SEQ ID NO:17	Kappa Light Chain Constant domain
SEQ ID NO:18	IgG1 Heavy Chain Constant domain
SEQ ID NO:19	GM285 Epitope 112-115
SEQ ID NO:20	GM285CDR 1 Heavy Chain
SEQ ID NO:21	GM285 CDR 2 Heavy Chain
SEQ ID NO:22	GM285 CDR 3 Heavy Chain
SEQ ID NO:23	GM285 CDR 1 Light Chain
SEQ ID NO:24	GM285 CDR 2 Light Chain
SEQ ID NO:25	GM285 CDR 3 Light Chain
SEQ ID NO:26	GM285 Heavy Chain Variable Domain
SEQ ID NO:27	GM285 Light Chain Variable Domain
SEQ ID NO:28	GM285 IgG1 Heavy Chain Constant domain
SEQ ID NO:29	GM285 Kappa Light Chain Constant domain
SEQ ID NO:30	GM37 Variant 1 Heavy Chain Variable Domain
SEQ ID NO:31	GM37 Variant 2 Heavy Chain Variable Domain
SEQ ID NO:32	GM37 Variant 3 Heavy Chain Variable Domain
SEQ ID NO:33	GM37 Variant 1 Heavy Chain CDR 2
SEQ ID NO:34	GM37 Variant 2 Heavy Chain CDR 2
SEQ ID NO:35	GM37 Variant 3 Heavy Chain CDR 2
SEQ ID NO:36	9E4 Binding Epitope
SEQ ID NO:37	Human Beta-Synuclein
SEQ ID NO:38	Human Gamma-Synuclein
SEQ ID NO:39	Alpha-Synuclein Ortholog for Cynomolgus Monkey
SEQ ID NO:40	Alpha-Synuclein Ortholog for Rat
SEQ ID NO:41	Alpha-Synuclein Ortholog for Mouse
SEQ ID NO:42	9E4 VH
SEQ ID NO:43	9E4 VL
SEQ ID NO:44	9E4 CDR 1 Heavy Chain

SEQ ID NO:45	9E4 CDR 2 Heavy Chain
SEQ ID NO:46	9E4 CDR 3 Heavy Chain
SEQ ID NO:47	9E4 CDR 1 Light Chain
SEQ ID NO:48	9E4 CDR 2 Light Chain
SEQ ID NO:49	9E4 CDR 3 Light Chain
SEQ ID NO:50	GM63 Epitope 126-138
SEQ ID NO:51	GM63CDR 1 Heavy Chain
SEQ ID NO:52	GM63 CDR 2 Heavy Chain
SEQ ID NO:53	GM63 CDR 3 Heavy Chain
SEQ ID NO:54	GM63 CDR 1 Light Chain
SEQ ID NO:55	GM63CDR 2 Light Chain
SEQ ID NO:56	GM63CDR 3 Light Chain
SEQ ID NO:57	GM63 Heavy Chain Variable Domain
SEQ ID NO:58	GM63 Light Chain Variable Domain
SEQ ID NO:59	GM63 IgG1 Heavy Chain Constant domain
SEQ ID NO:60	GM63 Kappa Light Chain Constant domain
SEQ ID NO:61	2E6 Epitope 126-140
SEQ ID NO:62	2E6 CDR 1 Heavy Chain
SEQ ID NO:63	2E6 CDR 2 Heavy Chain
SEQ ID NO:64	2E6 CDR 3 Heavy Chain
SEQ ID NO:65	2E6 CDR 1 Light Chain
SEQ ID NO:66	2E6 CDR 2 Light Chain
SEQ ID NO:67	2E6 CDR 3 Light Chain
SEQ ID NO:68	2E6 Heavy Chain Variable Domain
SEQ ID NO:69	2E6 Light Chain Variable Domain
SEQ ID NO:70	ch2E6 Heavy Chain Variable Domain
SEQ ID NO:71	ch2E6 Light Chain Variable Domain
SEQ ID NO:72	2E6 HLD 1 Heavy Chain Variable Domain
SEQ ID NO:73	2E6 HLD 1 Light Chain Variable Domain
SEQ ID NO:74	2E6 HLD 2 Heavy Chain Variable Domain
SEQ ID NO:75	2E6 HLD 2 Light Chain Variable Domain
SEQ ID NO:76	2E6 HLD 3 Heavy Chain Variable Domain
SEQ ID NO:77	2E6 HLD 3 Light Chain Variable Domain
SEQ ID NO: 78	D 1.2 CDR 1 Light Chain
SEQ ID NO:79	D 1.2CDR 2 Light Chain
SEQ ID NO:80	D 1.2CDR 3 Light Chain
SEQ ID NO:81	D 1.2CDR 1 Heavy Chain
SEQ ID NO:82	D 1.2 CDR 2 Heavy Chain
SEQ ID NO:83	D 1.2 CDR 3 Heavy Chain
SEQ ID NO:84	D 1.2 Light Chain Variable Domain
SEQ ID NO:85	D 1.2 Heavy Chain Variable Domain
SEQ ID NO:86	C 10.2 CDR 1 Light Chain
SEQ ID NO:87	C 10.2CDR 2 Light Chain
SEQ ID NO:88	C 10.2CDR 3 Light Chain
SEQ ID NO:89	C 10.2CDR 1 Heavy Chain
SEQ ID NO:90	C 10.2 CDR 2 Heavy Chain
SEQ ID NO:91	C 10.2 CDR 3 Heavy Chain

SEQ ID NO:92	C 10.2 Light Chain Variable Domain		
SEQ ID NO:93	C 10.2 Heavy Chain Variable Domain		
SEQ ID NO:94	CDR1 VL	7C4	
SEQ ID NO:95	CDR1 VL	7A10 &8D9	
SEQ ID NO:96	CDR3 VL	L3	
SEQ ID NO:97	CDR1 VH	7C4	
SEQ ID NO:98	CDR2 VH	5A1	
SEQ ID NO:99	CDR2 VH	9G11	
SEQ ID NO:100	CDR2 VH	9C12	
SEQ ID NO:101	CDR3 VH	5A1	
SEQ ID NO:102	CDR3 VH	9D7	
SEQ ID NO:103	CDR3 VH	7A10 &8D9	
SEQ ID NO:104	Full length VL	5A1	
SEQ ID NO:105	Full length VH	5A1	
SEQ ID NO:106	Full length VL	9D7	
SEQ ID NO:107	Full length VH	9D7	
SEQ ID NO:108	Full length VL	9G11	
SEQ ID NO:109	Full length VH	9G11	
SEQ ID NO:110	Full length VL	7C4	
SEQ ID NO:111	Full length VH	7C4	
SEQ ID NO:112	Full length VL	L3	
SEQ ID NO:113	Full length VH	L3	
SEQ ID NO:114	Full length VL	7A10	
SEQ ID NO:115	Full length VH	7A10	
SEQ ID NO:116	Full length VL	8D9	
SEQ ID NO:117	Full length VH	8D9	
SEQ ID NO:118	Full length VL	9C12	
SEQ ID NO:119	Full length VH	9C12	
SEQ ID NO:120	Full length VL	6B6	
SEQ ID NO:121	Full length VH	6B6	

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Further Embodiments of the Invention

1. An alpha-synuclein binding monoclonal antibody, or an antigen-binding fragment thereof, for use in inhibiting aggregation of tau.
- 5 2. The monoclonal antibody, or antigen-binding fragment thereof, according to item 1, wherein the antibody binds aggregated soluble forms of alpha-synuclein
3. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1 or 2, wherein inhibition of tau aggregation is *in vivo* or *in vitro*.
- 10 4. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items, wherein said alpha-synuclein antibody is administered to a patient with Alzheimer's disease.
5. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items, wherein the patient does not have Lewy body variant of Alzheimer's disease or combined Parkinson and Alzheimer's disease.
- 15 6. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-3 wherein said alpha-synuclein antibody is administered to a patient with a taupahty selected from the group comprising Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), Picks Disease, Primary age-related tauopathy (PART), Neurofibrillary tangle-predominant senile dementia, Dementia pugilistica, Chronic traumatic encephalopathy, stroke, stroke recovery, neurodegeneration in relation to Parkinson's disease, Parkinsonism linked to chromosome, Lytico-Bodig disease (Parkinson-dementia complex of Guam), Ganglioglioma and gangliocytoma, Meningio-angiomatosis, Postencephalitic parkinsonism, Subacute sclerosing panencephalitis, Huntington's disease, lead encephalopathy, tuberous sclerosis, Hallervorden-Spatz disease and lipofuscinosis. More typically, the taupathy is selected from the group consisting of Alzheimer's disease, Argyrophilic Grain Disease (AGD), Psychosis, particularly Psychosis due
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- to AD or Psychosis in patients with AD, psychiatric symptoms of patients with Lewy body dementia, Progressive Supranuclear Palsy (PSP), Frontotemporal dementia (FTD or variants thereof), TBI (traumatic brain injury, acute or chronic), Corticobasal Degeneration (CBD), and Picks Disease.
- 5 7. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items, wherein said alpha-synuclein antibody binds to the C-terminal part of alpha-synuclein
8. The monoclonal antibody, or antigen-binding fragment thereof, according to item 7, wherein the antibody binds to an epitope within the C-terminal
- 10 amino acids 110-140 of human alpha-synuclein.
9. The monoclonal antibody, or antigen-binding fragment thereof, according to items 7 or 8, wherein said epitope is within amino acids 112-117, 112-115, 118-126, 126-138 or 136-140 of human alpha-synuclein (**SEQ ID NO 10**).
- 15 10. The monoclonal antibody according to any one of the preceding items, wherein said antibody binds an epitope within amino acids 112-117 (**SEQ ID NO:9** (ILEDMP)) of human alpha-synuclein (**SEQ ID NO:10**), or antigen-binding fragment thereof that binds said epitope.
11. The monoclonal antibody, or antigen-binding fragment thereof, according to
- 20 according any one of the preceding items, wherein said antibody is capable of competing with an antibody comprising the light chain variable domain of **SEQ ID NO:8** and the heavy chain variable domain of **SEQ ID NO:7, 30, 31 or 32** for binding to said epitope.
12. The monoclonal antibody according to any one of the preceding items,
- 25 wherein said antibody is capable of specifically binding to an epitope within amino acids 112-115 (**SEQ ID NO:19** (ILED)) of human alpha-synuclein (**SEQ ID NO:10**), or antigen-binding fragment thereof that binds said epitope.

13. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items, wherein said antibody is capable of competing with an antibody comprising the heavy chain variable domain of **SEQ ID NO:26** and the light chain variable domain of **SEQ ID NO:27** for binding to said epitope
- 5
14. The monoclonal antibody according to item 9, wherein said antibody binds an epitope within amino acids 118-126 (such as **SEQ ID NO:36** (NEAYE)) of human alpha-synuclein (**SEQ ID NO:10**), or antigen-binding fragment thereof that binds said epitope.
- 10
15. The monoclonal antibody, or antigen-binding fragment thereof, according to item 9, wherein said antibody is capable of competing with an antibody comprising the heavy chain variable domain of **SEQ ID NO:42** and the light chain variable domain of **SEQ ID NO:43** for binding to said epitope.
- 15
16. The monoclonal antibody according to item 9, wherein said antibody binds an epitope within amino acids 126-138 (**SEQ ID NO:50** (EMPSEEGYQD YEP) of human alpha-synuclein (**SEQ ID NO:10**), or antigen-binding fragment thereof that binds said epitope.
- 20
17. The monoclonal antibody, or antigen-binding fragment thereof, according to item 9, wherein said antibody is capable of competing with an antibody comprising the heavy chain variable domain of **SEQ ID NO:57** and the light chain variable domain of **SEQ ID NO:58** for binding to said epitope.
- 25
18. The monoclonal antibody according to item 9, wherein said antibody binds an epitope within amino acids 126-140 (**SEQ ID NO:61** (EMPSEEGYQD YEPEA) of human alpha-synuclein (**SEQ ID NO:10**), or antigen-binding fragment thereof that binds said epitope.
19. The monoclonal antibody, or antigen-binding fragment thereof, according to item 18, wherein said antibody is capable of competing with an antibody comprising the heavy chain variable domain of **SEQ ID NO:68**

and the light chain variable domain of **SEQ ID NO:69** for binding to said epitope.

20. The monoclonal antibody according to any one of the preceding items comprising or consisting of an intact antibody.
- 5 21. The monoclonal antibody according to any one of the preceding items wherein the monoclonal antibody is selected from the group consisting of antibodies of subtype IgG1, IgG2, IgG3 or IgG4.
- 10 22. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items comprising or consisting of an antigen-binding fragment selected from the group consisting of Fv fragments (e.g. single chain Fv and disulphide-bonded Fv), Fab-like fragments (e.g. Fab fragments, Fab' fragments and F(ab)₂ fragments) and domain antibodies (e.g. single VH variable domains or VL variable domains).
- 15 23. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items wherein the antibody or antigen-binding fragment exhibits one or more of the following properties:
 - a) a binding affinity (KD) for alpha-synuclein of between 0.5-10 nM, such as 1-5 nM or 1-2 nM;
 - b) capability of inhibiting protease truncation of alpha-synuclein fibrils;
 - 20 c) capability of reversing impairment in basal synaptic transmission in F28-snca transgenic mice;
 - d) capability of reducing levels of alpha-synuclein in the mouse hippocampus as measured by in vivo microdialysis;
 - e) capability, when administered chronically, to restore motor function in a rat model of Parkinson's disease;
 - 25 f) Capability to prevent seeding of alpha-synuclein (such as accumulation of insoluble phosphorylated alphasynuclein in vitro and/or in a mouse model of Parkinson's disease); and/or
 - g) Capability to bind truncated alpha-synuclein in a human brain.

24. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the previous items that is a human, humanized, recombinant or chimeric antibody.
25. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-11 wherein the antibody, or a fragment thereof, comprises:
- 5 (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:2**;
- 10 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and
- 15 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**.
26. The monoclonal antibody, or antigen-binding fragment thereof, according to item 25, comprising the heavy chain variable domain of **SEQ ID NO:7**
- 20 or the light chain variable domain of **SEQ ID NO:8**.
27. The monoclonal antibody, or antigen-binding fragment thereof, according to item 25, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:7** and a light chain consisting of a variable domain of **SEQ ID NO:8**.
- 25 28. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-11 wherein the antibody, or a fragment thereof, comprises:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:33**;
- 30

- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
- 5 (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and
- (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**.
29. The monoclonal antibody, or antigen-binding fragment thereof, according
- 10 to item 28, comprising the heavy chain variable domain of **SEQ ID NO:30** or the light chain variable domain of **SEQ ID NO:8**.
30. The monoclonal antibody, or antigen-binding fragment thereof, according
- to item 28, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:30** and a light chain consisting of a variable domain of **SEQ**
- 15 **ID NO:8**.
31. The monoclonal antibody, or antigen-binding fragment thereof, according
- to items 1-11 wherein the antibody, or a fragment thereof, comprises:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
- 20 (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:34**;
- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
- 25 (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and
- (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**.

32. The monoclonal antibody, or antigen-binding fragment thereof, according to item 31, comprising the heavy chain variable domain of **SEQ ID NO:31** or the light chain variable domain of **SEQ ID NO:8**.
- 5 33. The monoclonal antibody, or antigen-binding fragment thereof, according to item 31, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:31** and a variable domain of **SEQ ID NO:8**.
34. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-11 wherein the antibody, or a fragment thereof, comprises:
- 10 (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:35**;
- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
- 15 (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and
- 20 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**.
35. The monoclonal antibody, or antigen-binding fragment thereof, according to item 34, comprising the heavy chain variable domain of **SEQ ID NO:32** or the light chain variable domain of **SEQ ID NO:8**.
- 25 36. The monoclonal antibody, or antigen-binding fragment thereof, according to item 34, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:32** and a variable domain of **SEQ ID NO:8**.
37. The monoclonal antibody, or antigen-binding fragment thereof, according to any items 1-13 wherein the antibody, or a fragment thereof, comprises:

- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:20**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:21**;
- 5 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:22**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:23**;
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:24**; and
- 10 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:25**.
38. The monoclonal antibody, or antigen-binding fragment thereof, according to item 37, comprising the heavy chain variable domain of **SEQ ID NO:26**
- 15 or the light chain variable domain of **SEQ ID NO:27**.
39. The monoclonal antibody, or antigen-binding fragment thereof, according to item 37, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:26** and a variable domain of **SEQ ID NO:27**.
- 20 40. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 16-17, wherein said alpha-synuclein antibody comprises:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:51**;
- 25 (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:52**;
- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:53**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:54**;
- 30

- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:55**; and
- (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:56**.
- 5 41. The monoclonal antibody, or antigen-binding fragment thereof, according to item 40, comprising the heavy chain variable region of **SEQ ID NO:57** or the light chain variable region of **SEQ ID NO:58**.
42. The monoclonal antibody, or antigen-binding fragment thereof, according to item 40, comprising the heavy chain variable region of **SEQ ID NO:57**
- 10 and the light chain variable region of **SEQ ID NO:58**.
43. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 14-15, wherein said alpha-synuclein antibody comprises:
- 15 (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:44**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:45**;
- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:46**;
- 20 (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:47**;
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:48**; and
- (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:49**.
- 25 44. The monoclonal antibody, or antigen-binding fragment thereof, according to item 43, comprising the heavy chain variable region of **SEQ ID NO:42** or the light chain variable region of **SEQ ID NO:43**.
45. The monoclonal antibody, or antigen-binding fragment thereof, according to item 43, comprising the heavy chain variable region of **SEQ ID NO:42**
- 30 and the light chain variable region of **SEQ ID NO:43**.

46. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- 5 ○ **SEQ ID NO: 94** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - 10 ○ **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - 15 ○ **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
47. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 46 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 94, 66 and 67**.
48. The monoclonal antibody, or antigen-binding fragment thereof, according to items 46 or 47, wherein said alpha-synuclein antibody comprises:
- 20 ○ **SEQ ID NO: 97** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - 25 ○ **SEQ ID NO: 63** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - 30 ○ **SEQ ID NO: 64** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.

49. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 48 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 97, 63 and 64**.
50. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 46 or 47 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 110**.
51. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 48 or 49 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:111**.
52. The monoclonal antibody, or antigen-binding fragment thereof, according to items 50 and 51 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:110** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:111**.
53. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- **SEQ ID NO: 95** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
54. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 53 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 95, 66 and 67**.

55. The monoclonal antibody, or antigen-binding fragment thereof, according to the preceding items 53 or 54 comprising a heavy chain variable region comprising the following CDRs:
- **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 63** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 103** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
56. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 55 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 63 and 103**.
57. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 53 or 54 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 114**.
58. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 55 or 56 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:115**.
59. The monoclonal antibody, or antigen-binding fragment thereof, according to items 57 and 58 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:114** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:115**.
60. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 53 or 54 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 116**.

61. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 55 and 56 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:117**.
- 5 62. The monoclonal antibody, or antigen-binding fragment thereof, according to items 60 and 61 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:116** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:117**.
- 10 63. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- 15 ○ **SEQ ID NO: 65** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - 20 ○ **SEQ ID NO: 96** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 25 64. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 63 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 65, 66 and 96**.
65. The monoclonal antibody, or antigen-binding fragment thereof, according to the preceding items 63 or 64 comprising a heavy chain variable region comprising the following CDRs:
- 30 ○ **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;

- **SEQ ID NO: 63** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - 5 ○ **SEQ ID NO: 64** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 66. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 65 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 63 and 64.**
- 67. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 63 or 64 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 112.**
- 15 68. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 65 or 66 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:113.**
- 69. The monoclonal antibody, or antigen-binding fragment thereof, according to items 67 and 68 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:112** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:113.**
- 20 70. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- 25
 - **SEQ ID NO: 65** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - 30 ○ **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid

- differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
- **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 5
71. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 70 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 65, 66 and 67.**
- 10
72. use according the preceding items 70 or 71 comprising a heavy chain variable region comprising the following CDRs:
- **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 98** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 101** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 15
- 20
73. The use according to Item 72 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 98 and 101**
- 25
74. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 70 or 71 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 104.**
75. The use Items 72 or 73 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:105.**
- 30

76. The monoclonal antibody, or antigen-binding fragment thereof, according to items 74 and 75 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:104** and heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:105**.
77. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- **SEQ ID NO: 65** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
78. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 77 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 65, 66 and 67**.
79. The monoclonal antibody, or antigen-binding fragment thereof, according to the preceding items 77 or 78 comprising a heavy chain variable region comprising the following CDRs:
- **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;

- **SEQ ID NO: 99** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - 5 ○ **SEQ ID NO: 64** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 80. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 79 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 99 and 64**.
- 10 81. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 77 or 78 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 108**.
- 15 82. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 79 or 80 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:109**.
- 20 83. The monoclonal antibody, or antigen-binding fragment thereof, according to items 81 and 82 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:108** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:109**.
- 25 84. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
 - **SEQ ID NO: 65** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - 30 ○ **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid

- differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
- **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 5
85. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 84 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 65, 66 and 67.**
- 10
86. The monoclonal antibody, or antigen-binding fragment thereof, according to the preceding items 84 or 85 comprising a heavy chain variable region comprising the following CDRs:
- **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 100** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 64** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 15
- 20
- 25
87. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 86 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 100 and 64.**
88. The use according to Items 84 or 85 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 118.**
- 30
89. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 86 or 87 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 119.**

90. The monoclonal antibody, or antigen-binding fragment thereof, according to any one of the preceding items 88 and 89 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:118** and heavy a chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:119**.
91. The monoclonal antibody, or antigen-binding fragment thereof, according to items 1-9 and items 18-19, wherein said alpha-synuclein antibody comprises:
- **SEQ ID NO: 65** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;
 - **SEQ ID NO: 66** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 67** or an amino acid sequence having with no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
92. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 91 comprising a light chain variable region comprising the CDRs of **SEQ ID NOs 65, 66 and 67**.
93. The monoclonal antibody, or antigen-binding fragment thereof, according to the preceding items 91 or 92 comprising a heavy chain variable region comprising the following CDRs:
- **SEQ ID NO: 62** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference;

- **SEQ ID NO: 63** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference; and
 - **SEQ ID NO: 102** or an amino acid sequence having no more than 4 amino acid differences, or no more than 3 amino acid differences, or no more than 2 amino acid differences, or no more than 1 amino acid difference.
- 94. The monoclonal antibody, or antigen-binding fragment thereof, according to Item 93 comprising a heavy chain variable region comprising the CDRs of **SEQ ID NOs 62, 63 and 102**
- 95. The monoclonal antibody, or antigen-binding fragment thereof, according to Items 91 or 92 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO: 106**.
- 96. The antibody or antigen-binding fragment thereof according to Items 93 or 94 comprising a heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:107**.
- 97. The monoclonal antibody, or antigen-binding fragment thereof, according to items 95 or 96 comprising a light chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:106** and heavy chain variable region comprising or consisting of the amino acid sequence of **SEQ ID NO:107**.
- 98. A nucleic acid encoding the antibody or the fragment according to any one of items 25-97.
- 99. A pharmaceutical composition comprising the monoclonal antibody, or an antigen-binding fragment thereof, according to any one of the previous items or the preparation of any one of items 25-97, and a pharmaceutical acceptable carrier.
- 100. A method of treating a disease according to items 5 or 6 in a subject, said method comprising administering the monoclonal antibody or antigen-binding fragment thereof of any of items 1-97 to said subject in an effective amount.

101. The method according to item 100, wherein the treatment is chronic
102. The method according to item 101, wherein the chronic treatment is for at least 2 weeks.
- 5 103. The method according to item 100, wherein the subject is human.
104. A kit comprising the antibody, or antigen-binding fragment thereof, according to items 1-97 for use in a method according to item 100.
105. The monoclonal antibody, or antigen-binding fragment thereof, according to items 5 or 6, wherein the monoclonal antibody of items 1-97 is detectably labelled.
- 10 106. The monoclonal antibody, or antigen-binding fragment thereof, according to of item 105, wherein said detectable label is a fluorescent label, a chemoluminescent label, a paramagnetic label, a radioisotopic label or an enzyme label.
- 15 107. The monoclonal antibody, or antigen-binding fragment thereof, according to of items 105-106 for use in detecting or measuring the presence or amount of said alpha-synuclein in the brain or any other organ or body fluid of a subject.
- 20 108. The monoclonal antibody, or antigen-binding fragment thereof, according to items 105-107, wherein said detection or measurement comprises in vivo imaging of said anti-synuclein antibody bound to said alpha-synuclein.
- 25 109. The monoclonal antibody, or antigen-binding fragment thereof, according to items 105-108, wherein said detection or measurement comprises ex vivo imaging of said anti-synuclein antibody or said antigen-binding fragment thereof, bound to said alpha-synuclein.
- 30 110. Use of a monoclonal antibody, or antigen-binding fragment thereof, according to any one of items 1-97 for use in the manufacturing of a medicament for treating, diagnosing or imaging a disease according to items 5 or 6.
111. A method of delaying the progression of a disease according to items 5 or 6 in a patient, said method comprising reducing or attenuating

the accumulation of pathological tau protein in said patient by administering an antibody as defined in items 1-97

EXAMPLES

Example 1: Antibody Screening

1. Immunogen and ligand production

[000135] The following proteins were acquired or produced for use as immunogens shown in Fig 1. The mice were immunized with three immunogens: full length recombinant human alpha-synuclein fibrils; human alpha-synuclein recombinant protein containing amino acids 1-60 (Rpeptide, Bogart, Georgia) and human alpha-synuclein recombinant protein containing amino acids 1-119. To make the fibrils from the full length the alpha-synuclein a lyophilized product from Rpeptide, Bogart, Georgia (Catalog number S-1001-2) was used. This was dissolved in 20 mM tris and 300 mM NaCl buffer at concentration of 1 mg/ml protein. To make the fibrils the protein solution was incubated 170 µl aliquots in 96 well plate with a 70 µm diameter ceramic bead in each well at 200 rpm in Vortemp 56 shaker incubator (Labnet International, Edison, NJ, USA), at 37 °C for 7 days, and the formation of fibrils was followed by adding thioflavin T and measuring fluorescence in one of the wells. The recombinant alpha-synuclein containing amino acids 1-60 was dissolved in water to give a concentration of 1 mg/ml.

[000136] The recombinant alpha-synuclein containing amino acids 1-119 was made using the following construct: A synthetic gene coding for a 6 amino acid Histidine tag, followed by factor Xa cleavage site and sequence coding for human alpha-synuclein amino acids 1-119:

MAHHHHHHIE GRMDVFMKGL SKAKEGVVAA AEKTKQGVAE AAGKTKEGVL
YVGSKTKEGV VHG VATVAEK TKEQVTNVGG AVVTGVTAVA QKTVEGAGSI
AAATGFVKKD QLGKNEEGAP QEGILEDMPV D **(SEQ ID NO:16)**

was synthesized by Genscript and cloned into NdeI-XhoI site in pET24a(+) expression vector (Novagen).

[000137] The expression vector was transformed into E. coli BL21 and a single colony picked for expression using the overnight express autoinduction system from Novagen (User protocol TB383 rev. H1005). The scale was 500 ml of final culture volume. Cells were harvested by centrifugation 10 min at 6000g

and subsequently lysed using BugBuster protein extraction Reagent (User protocol TB245 Rev. 0304). After lysis the sample was cleared by centrifugation and the supernatant used for further purification.

[000138] The His-tagged protein was purified on a 5ml HisTrap column (GE healthcare) equilibrated in 20 mM Sodium phosphate pH7.5, 1 M NaCl (A-buffer). After sample application and wash using A-buffer the protein was eluted in a gradient to 0.25 M Imidazole in A-buffer over 20 column volumes. Fractions of 5 ml were collected and analyzed by SDS-PAGE. Fractions with the protein of interest was pooled, concentrated and applied to an S200 (26/60) size exclusion column (GE healthcare) in 10 mM tris pH 7.4, 300 mM NaCl. Again fractions were pooled according to presence in SDS-PAGE of a band with expected size.

[000139] To remove the N-terminal tag, the purified his-tagged alpha-synuclein 1-119 was incubated with factor Xa in a 1:50 ration using the Novagen kit (69037-3FRX). After overnight incubation, the factor Xa was removed batchwise using Xarrest agarose. The cleaved alpha-synuclein 1-119 was finally purified by permissive HisTrap chromatography as described above. From the flow through the purified alpha-synuclein 1-119 was obtained and concentrated to ~400 µg/ml using centricon concentration devises.

[000140] Alpha-synuclein (Rpeptide) was rehydrated in PBS at 2 mg/ml and peroxyntirite (100µL/mg protein) was added dropwise while mixing. The nitrosylated alpha-synuclein was then dialyzed in 5L PBS and stored at -20°C.

[000141] Dopamine was used to oxidize alpha-synuclein. Equal volumes of a 200uM solution of Dopamine-HCL (Sigma P5244) prepared in 10mM PBS, pH7.4 and a 28µM solution of alpha-synuclein (Rpeptide) in 10mM PBS, pH7.4 were combined. The resulting 14 uM alpha-synuclein/100 uM Dopamine were incubated at 37°C O/N (over night). The oxidized alpha-synuclein was then dialyzed in PBS and stored at -20°C.

[000142] Different native and chimeric versions of synuclein proteins were produced in order to screen a diverse library of anti-alpha-synuclein antibodies.

Screening constructs included the following: human, mouse, rat and cynomolgus monkey alpha-synuclein, human Beta-synuclein, Human Gamma-synuclein and lastly an alpha-synuclein derivative that lacked residues 120-140 of alpha-synuclein. In addition, a series of 4 shuffle constructs: A-Syn-AAKK-BAP, A-Syn-BAAK-BAP, A-Syn-BBAA-BAP, A-Syn-BBKK-BAP (**SEQ ID Nos:11-14**) were produced. These constructs contained linear stretches of human alpha-synuclein (A), human Beta-synuclein (B) and chicken alpha-synuclein (K). Gene were cloned containing a Biotin Acceptor Peptide (BAP) tag fused to the C-terminus of the ligands in order to facilitate site specific biotinylation of each of the ligands. The biotinylation allowed for attachment of the ligands to beads used in the soluble ELISA format. Mammalian expression vectors were constructed carrying the different alpha-synuclein BAP tag fusion constructs (ASynBAP). The ligands were expressed in HEK 293 cells using transient transfection (Genmab A/S).

2. Immunization

[000143] Antibodies HuMab-Synuclein were derived from the immunizations of HuMab mouse strain HCo17-BALB/c and HCo12-BALB/c mice, double knock out for the mouse immunoglobulin (Ig) heavy and mouse kappa light chain, which prevents the expression of antibodies that are completely murine (human monoclonal antibody; Medarex Inc., San Jose, CA, USA). The various mouse strains were made transgenic by the insertion of human Ig heavy and human Ig kappa light chain loci and differ in the number of human VH (variable domain of heavy chain) and VL (variable domain of light chain) genes.

[000144] 48 Mice were immunized alternating intraperitoneally (IP) with 20 µg antigens and subcutaneously (SC, at the tailbase) with the same immunogen, with an interval of 14 days. A maximum of eight immunizations were performed, 4 IP and 4 SC.

[000145] The first immunization was performed with alpha-synuclein immunogens in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA), the following immunizations in incomplete Freund's adjuvant (IFA). When serum titers were found to be sufficient (dilution of serum of 1/50 or lower found

positive in antigen specific screening assay as described in herein above on at least two sequential, biweekly, screening events), mice were additionally boosted twice intravenously (IV) with 10 µg alpha-synuclein immunogen protein in 100 µL PBS, four and three days before fusion.

5 **[000146]** The immunization protocols are shown in **Fig 1**.

[000147] Antibody GM37 came from an immunization protocol where human full length α -Synuclein-fibrils was used, alternating with alpha-synuclein C- terminally truncated forms with amino acids 1-60 and 1-119.

[000148] Antibody GM285 came from an immunization protocol where
10 Human α -Synuclein-monomer 1-140 was used for the first 4 immunizations. If there was no titer, the immunization was continued with fibrils (ip/sc), otherwise it was continued with monomer.

3. HuMab hybridoma generation

[000149] HuMab mice with sufficient antigen-specific titer development as
15 defined above were sacrificed and the spleen and lymph nodes flanking the abdominal aorta and caval vein were collected. Fusion of splenocytes and lymph node cells with a mouse myeloma cell line was done by electrofusion using a CEEF 50 Electrofusion System (Cyto Pulse Sciences, Glen Burnie, MD, USA), essentially according to the manufacturer's instructions. Fused cells were seeded
20 in fusion medium containing 10% Fetal Clone I Bovine serum (Perbio), 1 mM sodium pyruvate (Cambrex), 0.5 U/mL penicillin, 0.5 U/mL streptomycin (Cambrex), 50 µM 2-mercaptoethanol (Invitrogen), 600 ng/mL interleukin 6 (IL-6) (Strathmann), 1 x HAT (Sigma) and 0.5 mg/mL kanamycin (Invitrogen) in HyQ mADCF-Mab (Perbio). After ten days, supernatant was harvested and cells were
25 refreshed with harvest medium, containing 10 % Fetal Clone I Bovine serum, 0.5 U/mL penicillin, 0.5 U/mL streptomycin, 600 ng/mL IL-6 and 1 x proHT (Cambrex) in HyQ mADCF-Mab. Supernatants of the hybridoma cultures were screened by primary screening assays. Supernatants were characterized for binding to eight different ligands. These included 4 orthologs: human, mouse, rat and
30 cynomologus monkey, human α -synuclein Beta-synuclein and human

Gamma-synuclein (**SEQ ID NOs 37-41**) and lastly they were tested for their ability to bind to a human alpha-synuclein derivative that lacked residues 120-140 of alpha-synuclein.

- 5 **[000150]** The screening of anti-alpha-synuclein antibodies was performed using a high throughput suspension ELISA format using automated liquid handling systems (Genmab A/S). The reading of the plates was performed by two systems, the FMAT 8200 from Applied Biosystems was used to read 384 well plates and the ImageXpress Velos Cytometer from Molecular Devices was used to read the 1536 well plates.
- 10 **[000151]** In the primary screen clones were characterized by their ability to bind 8 different ligands. These included a series of 4 shuffle constructs: A-Syn-AAKK-BAP, A-Syn-BAAK-BAP, A-Syn-BBAA-BAP, A-Syn-BBKK-BAP (**SEQ ID NOs:11-14**), alpha-synuclein 120-140 deletion-BAP, nitrated human alpha-synuclein-BAP and oxidized human alpha-synuclein-BAP.
- 15 **[000152]** In short, the sera or supernatant potentially containing alpha-synuclein specific antibodies were added to the beads to allow binding to alpha-Synuclein and/or alpha-synuclein derived constructs. The binding of the anti-alpha-synuclein antibodies is detected using a fluorescent conjugate, DyLight649 conjugated goat antihuman IgG, Fc specific. Two known mouse anti-alpha-synuclein antibodies, LB509 and Syn211, were included in screenings as positive controls. To ensure specific detection of alpha-synuclein antibodies, an anti-alpha-synuclein sera pool was used as a negative control in the 384 well format titer screening while human ChromPure IgG is used in the 1536 well format 8-bead based assay.
- 20 **[000153]** Hybridoma cells from the best primary wells were seeded in semisolid medium made from 40% CloneMedia (Genetix, Hampshire, UK) and 60% HyQ 2x complete medium (Hyclone, Waltham, USA). For each primary well, a well of a Genetix black 6-well plate was seeded. From each well, 25 sub clones were picked, using the ClonePix system (Genetix). The sub clones were picked in
- 25 **[000153]** harvest medium. After seven days, the supernatants of the sub clones were
- 30

screened again for Synuclein-specific human IgG binding and the human IgG concentration was measured using Octet (ForteBio, Menlo Park, USA). From each primary well, the best sub clone was selected and expanded in expansion medium containing only 600 ng/mL IL-6, 0.5 U/mL penicillin, 0.5 U/mL streptomycin and 1 x proHT. The sub clones were expanded from one 96-well plate well to one 24-well plate well to four 24-well plate wells to six 6-well plate wells. Clones derived by this process were designated as primary clones (PC).

[000154] Additional antibody binding studies were performed using Octet 384RED (ForteBio, Menlo Park, USA). HuMab antibody solutions of 2 µg/ml were made by dilution in sample diluent (ForteBio, art. No. 18-5028). Amine reactive sensors (ForteBio, art.no. 18-0008) were used for immobilization of HuMabs. Prior to coupling to amine reactive sensors, HuMabs were diluted in MES pH 6.0 buffer (18-5027). Coupling was performed at 30°C and 1000 rpm as follows: Amine reactive sensors were pre-wet in PBS and subsequently activated with EDC/NHS(ForteBio. Art.no. 18-1033/18-1034) activation solution (according to manufacturer's instruction) for 300 seconds. Activated sensors were immobilized with HuMabs during 600 seconds.

[000155] The binding of GM37 and GM285 in Octet to recombinant human, cynomolgus and mouse alpha-synuclein, and lack of binding to human beta or gamma-synuclein is shown in **Figure 2**.

4. Sequence analysis of the Synuclein-specific HuMab variable domains and cloning in expression vectors

[000156] Total RNA was prepared from 0.2 to 5x10⁶ hybridoma cells and 5'-RACE-Complementary DNA (cDNA) was prepared from 100 ng total RNA, using the SMART RACE cDNA Amplification kit (Clontech). VH and VL coding regions were amplified by PCR and cloned directly, in frame, in the p33G1f and p33Kappa expression vectors (containing the human IgG1/kappa constant domain encoding sequences), by ligation independent cloning (Aslanidis, C. and P.J. de Jong, Nucleic Acids Res 1990;18(20): 6069-74). For each antibody, 16 VL clones and 16 VH clones were sequenced. Clones with a correct Open Reading Frame (ORF) were selected for further study and expression. Vectors of

all combinations of heavy chains and light chains were transiently co-expressed in Freestyle™ 293-F cells using 293fectin.

5 **[000157]** In the case of GM37 sequencing of the VH region identified an extra cysteine in the CDR3 domain at position 106. In order to eliminate the possibility of misfolding and potential loss of antibody activity due to disulfide bond formation the cysteine was mutated to serine at position 106.

[000158] Comparator antibody 9E4 was generated based on the VH and VL sequence derived from hybridoma PTA-8221 (US patent 20080175838) (**SEQ ID NO 42 and 43**)

10 **5. Expression/Purification of Antibodies**

15 **[000159]** Antibodies were produced by transfection in HEK293 6E cells using the pTT5 vectors and PEIpro as a transient transfection agent (National Research Council of Canada). In short, The heavy and light chains were transfected into HEK293 cells using PEIpro (VWR), and cells were supplemented with TN1 (Sigma) 24 hours after transfection. Cells were grown until the viability approached 50%, and yield of antibody measured by easy IgG titre (Thermo). Culture supernatant was filtered over 0.2 µm dead-end filters, loaded on 5 mL Protein A columns (rProtein A FF, Amersham Bioscience) and eluted with 0.1 M citric acid-NaOH, pH 3. The eluate was immediately neutralized with 2M Tris-HCl, 20 pH 9 and dialyzed to 12.6 mM NaH₂PO₄, 140 mM NaCl, pH 7.4 (B.Braun), O/N. After dialysis, samples were sterile-filtered over 0.2 µm dead-end filters. Purity was determined by SDS-PAGE and concentration was measured by nephelometry and absorbance at 280 nm. Purified antibodies were aliquoted and stored at -80°C.

25 **Example 2: Antibody Characterization Using Surface Plasmon Resonance**

[000160] Real time binding of the antibodies to alpha-synuclein was measured using a BIAcore® 3000. A capture surface was prepared by amine-coupling a polyclonal rabbit Anti-Mouse antibody (part of Mouse Antibody Capture Kit, GE Healthcare, Cat. no: BR-1008-38) in first flow cell (Fc1) and

second flow cell (Fc2) of a CM5 chip (BIAcore®). The mouse antibody was captured in Fc2 at the concentration required to achieve a ligand level of around 500RU. The baseline was allowed to stabilize for 10min before injecting analyte (ASynBAP) in Fc1-2 at 30µl/min. ASynBAP was run at 100-3200nM and 25-
5 3200RU, respectively. The highest concentration in each titration series was run in duplicate. The surface was regenerated with 10mM Glycine-HCl, pH 1.7 (30sec inject) to remove captured mouse antibody and analyte in the end of each cycle. HBS-EP (GE Healthcare, Cat. No: BR-1001-88) was used as running buffer and sample diluent in all experiments and the assay was run at 25°C. All
10 samples were kept at 4°C before acquisition.

[000161] The response recorded in Fc1, where capture antibody had been immobilized but no Alpha-Synuclein antibody captured, was subtracted from the response in Fc2. A 1:1 or 2:1 binding algorithm was fit to the dataset using BIAevaluation software version 4.1.1. Results can be seen in **Figs. 3, 4 and 5**
15 showing binding of antibody GM37, GM285 and 9E4 to human alpha-synuclein.

Example 3: Epitope Mapping

[000162] Epitope mapping of the antibodies to alpha-synuclein was done with arrays of overlapping linear peptides at Pepscan (Pepscan Zuidersluisweg 2 8243 RC Lelystad, The Netherlands). The binding of antibody to each of the
20 synthesized 20 mer peptides was tested in a Pepscan based ELISA. The linear peptide array covering the entire coding sequence of alpha-synuclein, as well as all peptides with oxidized methionines or nitrosylated tyrosines, were incubated with primary antibody solution (overnight at 4°C). After washing, the peptide arrays were incubated with a 1/1000 dilution of an antibody peroxidase conjugate
25 (SBA, cat. nr. 2010-05) for one hour at 25°C. After washing, the peroxidase substrate 2,2'-azino-di-3-ethylbenzthiazoline sulfonate (ABTS) and 2 µl/ml of 3 percent H2O2 were added. After one hour, the color development was measured. The color development was quantified with a charge coupled device (CCD) - camera and an image processing system. For data processing the values were
30 obtained from the CCD camera range from 0 to 3000 mAU, similar to a standard 96-well plate ELISA-reader. The results were quantified and stored into the

Peplab database. Occasionally a well contains an air-bubble resulting in a false-positive value, the cards are manually inspected and any values caused by an air-bubble are scored as 0. The binding data of antibody GM37 and GM285 to peptides containing the sequence ILEDMP or ILED respectively can be seen in

5 **Figure 6.**

Example 4: GM37 and GM37 variants

10 **[000163]** The anti-alpha-synuclein antibodies were produced in mammalian cell culture under conditions that mimic the production conditions that will be used for producing clinical grade material for use in patients. It is well known that proteins produced in this manner undergo post-translational modifications that can impact both therapeutic potency of the antibody as well as biophysical attributes that affect the stability of the antibody over time. Empirical knowledge ascertained from decades of studies identified a set of post-translational modifications known to provide risk for the developability of a specific molecule. These post-translational modifications have been shown to correlate with amino acid strings present in the primary sequence of the heavy and light chain proteins. Algorithms have been generated that can identify these sequences and determine the potential risk they will have on the manufacturability and developability of a therapeutic antibody.

20 **[000164]** In silico analysis of the primary sequence of the antibody can be used to de-risk a molecule for its potential to be developed as a therapeutic. In particular, detailed analysis of the VH and VL regions can identified unique amino acids that are deemed important for the molecules activity but also may be a potential risk for its stability over time. Sequence specific deamidation has been identified as a potential risk for protein structures. Protein deamidation can occur on the amide side chains of glutamines or asparagine residues and transform them into a carboxylate group (Lorenzo et al. PLOSone, DOI:10.1371, Dec. (2015)). Nonenzymatic deamidation at neutral pH occurs faster for asparagine and is therefore considered a higher risk than glutamine. The activity is further influenced by the subsequent amino acid in the sequence and can occur at a rate of days or years. The

actual fate of the protein that undergoes deamidation needs to be evaluated experimentally to determine the impact of the change both on its stability and activity.

[000165] We identified a site for deamidation within the VH domain of
5 GM37. Amino acid residues 54 is an asparagine(N) followed by a glycine(G)
at position 55. The N54 is at high risk for spontaneous deamidation. To
mitigate this risk we generated a set of 3 variants that replace the
asparagine(N) with serine(S), glutamine(Q) or histidine(H). All 3 variants
were produced in mammalian cell culture using transient transfection
10 methods. All 3 variants showed similar expression and purification properties
as GM37wt.

[000166] For each of the eight products 400 ml transient transfections were
performed using CHOK1SV GS-KO cells which had been in culture for
minimum 2 weeks. Cells were sub-cultured 24 hours prior to transfection. All
15 transfections were carried out via electroporation using Gene Pulse XCell
(Bio-Rad). For each transfection, viable cells were resuspended in pre-
warmed CD-CHO media supplemented with 6mM L-glutamine to 2.86×10^7
cells/ml. 40 µg of each established SGV DNA containing the appropriate
heavy and light chains were aliquoted into each cuvette (Bio-Rad,
20 GenePulser cuvette, 0.4 cm gap, 165-2088) and 700 µl cell suspension
added. Cells were electroporated at 300V, 900µF. Transfected cells were
transferred to rep-warmed media in Erlenmeyer flasks and the contents of the
cuvettes rinsed twice with prewarmed media were also transferred to the
flasks. Transfectant cultures were incubated in a shaking incubator at 36.5°C,
25 5% CO₂, 85% humidity, 140 rpm for 6 days. Cell viability was measured at the
time of harvest using a Cedex HiRes automated cell counter (Rosche).

[000167] In order to evaluate the importance of residue 54 in binding to
human alpha-synuclein we analyzed the ability of the variants to bind in two
different experiments. Using a competition ELISA format we evaluated the
30 impact the change at residue 54 would have on the ability of GM37 to bind
alpha-synuclein in solution. By evaluating the concentration of synuclein able

to inhibit binding of the antibody to synuclein coated ELISA plates we showed all three variants maintained the same binding as GM37wt and bind to alpha-synuclein with high affinity resulting in IC50s of 1-2nM (**Fig. 7**). A competition assay was performed using preincubation of a fixed concentration (0.3 µg/ml) of each of the following antibodies, GM37 (named GM 37wt), GM37 variant1, GM37 variant2 and GM37 variant3 with a range of 0-1000 nM human alpha-synuclein for 60 minutes at room temperature. The remaining unbound antibody was captured and measured on ELISA plates coated with 100 ng/ml of recombinant human alpha-synuclein using an anti-human detection antibody by electrochemiluminescence (MSD, Gathersburg, MD). The IC50s of the interaction are 1.9nM, 1.6nM, 2.1nM and 1.4nM for GM37 wt, GM37variant1, GM37variant2 and GM37variant3, respectively (as determined using Prism Graphpad®).

[000168] Using surface plasmin resonance (SPR), we evaluated the real time kinetics of binding of GM37 wt (2 batches) and the three variants (**Example 2**). The human alpha-synuclein was captured to the slide(ligand) and the antibodies were each tested at multiple concentrations as analytes. Analysis of the binding curves in the presence of antibody at multiple concentrations showed that the on rates were the same for all four antibodies, similarly when the antibody was removed from the buffer the off-rates measured showed no statistical difference between the antibodies. Using a 1:1 binding algorithm all 4 antibodies have near identical binding constants (**Fig. 7**). concern over the loss of potency.

Example 5: Tau aggregation induced by alpha-synuclein seeds can be prevented by antibodies against alpha-synuclein

Description of alpha-synuclein fibril (seed) preparation

Fibrillation of alpha-synuclein can be done following slightly different protocols.

Recombinant alpha-synuclein purchased from rPeptide (catalog # S-1001-2) was dissolved according to the manufacturers recommendation in double-distilled water resulting in a 1 mg/ml solution in 20 mM Tris-HCL/100 mM NaCl, pH = 7.4. Alpha-synuclein preformed fibrils (PFFs) were generated from monomeric alpha-synuclein using the Virginia Lee/Kelvin Luk protocol (Luk et al, Science, 2012, 16;338(6109):949-53). The 1 mg/ml solution was incubated at 37°C with agitation (300 rpm) for 2 days, then a pause for 3 days, then 1 day of agitation, then 1 day pause, then 4 days of agitation. After that the fibrils were harvested and kept at -20°C until use. When fibrils were used in cellular assays, they were always sonicated at 5 min, setting 5.50% cycle, with horn probe sonicator, immediately prior to addition.

Alternatively the 1 mg/ml solution is shaken constantly for 5-7 days at 37°C. The end product is termed "crude fibrils". Upon sonication, they are termed "crude seeds". The crude fibrils can be centrifuged and the pellet containing the aggregated alpha-synuclein is suspended in fresh PBS, and called "pure fibrils". "Pure seeds" are obtained by sonicating pure fibrils.

Alpha-synuclein antibody-mediated inhibition of seeding of Tau

In order to show the effect of alpha-synuclein antibody mediated inhibition of seeding of intracellular Tau, a HEK293 cell based seeding assay was setup (Figure 8, lower panel illustrates the assay set-up). HEK293 cells were plated 100,000 cells /well in 24 well plates and transiently transfected with cDNA encoding human tau-P301L-FLAG in 24 hours after plating. Twenty four hours after transfection cells were seeded (using Lipofectamine2000 transfection) with aggregated fibrillated alpha-synuclein (seeds) with or without antibodies for 24 hours, followed by splitting and re-plating cells and harvesting after additional 24 hours. Cells were lysed and sonicated in PBS, supplemented with 1% triton X, phos-stop and complete phosphatase and protease inhibitors (Roche) buffer. Total cell lysates were analyzed using the tau aggregation assay from Cisbio. This assay is based on time-resolved fluorescence using the same antibody for both donor (Tb3+ conjugated) and acceptor (d2 conjugated) antibody in FRET. A 10

5 μ l sample was mixed with 10 μ l antibody mix and incubated for 20h. The plate was read on the Pherastar plate reader to assess time-resolved fluorescence (FRET signal measured/integrated after switching of the excitation light). The assay measures aggregated tau both in human brain autopsy material from AD patients, brain material from Tau transgenic mice (rTg4510) and in seeded HEK293 cells with high specificity and sensitivity. Using different preparations of fibrillated Tau protein as seed this type of HEK293 cell based seeding assay has been efficiently used to select Tau antibody clinical candidates.

10 Results are shown in Figure 8, upper panel. Transfection of alpha-synuclein seeds (300ng crude seeds) alone results in a relative tau aggregation around 100 (alpha-Syn, 3rd bar) indicating that alpha-synuclein potentially induce cross-seeding of endogenous Tau. The seeding effect was not affected by co-transfection with B12 (control antibody) and the following Tau antibodies (mC10-2, mD1.2, an tau binding antibody named LU0041G and humanized (h) C10-2). By 15 co-incubating the cells with four different antibodies against alpha-synuclein HLD1, GM37 (37), GM63 (63) and 9E4 there was however a partial reversal of Tau aggregation, For example the antibody 9E4 results in an increase in relative aggregation to 20 (as compared to 100 in non-treated controls). All antibodies were co-transfected with crude seeds (2.4 ug antibody and 300 ng seeds in a total volume of 400ul). 20

Example 6: Antibody Discovery 2E6 and 2E6 variants

A. Immunization/Hybridoma screening

25 Monoclonal antibodies against alpha-synuclein were generated by immunizing mice with different synuclein aggregates cross linked with for example reactive aldehydes. The first antigen was made of recombinant lyophilized alpha-synuclein from Rpeptide (4241 Mars Hill Road, Bogart, GA 30622, USA). It was made by dissolving the protein in PBS to give a solution of 70 uM alpha-synuclein (1mg/ml). 30 The solution was incubated 18 hours at 37 degrees C and frozen in 100 ul aliquots. The second antigen was made similarly from recombinant alpha-synuclein (Rpeptide) by dissolving it at 70 microM in 20 mM Tris (pH=7.4), 0.15 M NaCl. Reactive

aldehyde ONE (4-oxo-2-Nonenal, Cat # 10185 from Cayman Chemicals, Ann Arbor, MI) was added in a molar ratio of 20:1 to covalently cross link oligomers of alpha-synuclein. The solution was incubated for 18 hours at 37 C (without shaking). The unreacted ONE was removed by Vivaspin500 spin column (10 kDa MWCO) and the samples were dialyzed against 20 mM Tris, pH 7.4, 0.15 M NaCl, and frozen in aliquots. The third antigen was recombinant alpha-synuclein fragment amino acids 1-60 (Rpeptide) which was sent to as lyophilized powder (original material from Rpeptide). Briefly, three female mice (4–7 weeks old) were immunized and boosted up to three times. Tail-bleeds were taken and screened for anti-synuclein antibodies by enzyme-linked immunosorbent assay (ELISA) against the antigen. Titer is defined by the serum dilutions to achieve OD reading of 3-times the base line in an ELISA. Mice showing a titer greater than 1:50,000 over control were selected for fusion. Harvested splenocytes were fused to SP2/0 mouse myeloma cells, diluted and plated from single cell fusions. Supernatants were harvested 14 days post-fusion and screened for antibody production. Using the synuclein ELISA 50 positive clones were recovered from ~1000 wells. A Clonotyping System/AP kit was used for immunoglobulin isotyping (Southern Biotechnology, Birmingham, AL). The 50 anti-alpha-synuclein supernatants were screened for reduction of accumulation of atto-labelled alpha-synuclein aggregates in the SKMEL5 cell assay below. The commercial antibody LB509 was included as positive control. It was found that out of the 50 antisera, only 4 antisera reduced the intracellular accumulation of alpha-synuclein and these antibodies were taken forward for cloning. These four antibodies were then tested in dose response in the assay. The antibody with largest effect, 2E6, was selected for further characterization in PD relevant models.

Description of fibril preparation

Recombinant alpha-synuclein was ordered from rPeptide (catalog # S-1001-2) and dissolved according to the manufacturers recommendation in double-distilled water resulting in a 1 mg/ml solution in 20 mM Tris-HCL/100 mM NaCl, pH = 7.4. The alpha-synuclein was fluorescently labelled with Atto488 by using the Atto488 Protein Labeling Kit from Sigma (#38371). A mixture of 30 % Atto488-labelled

and 70 % unlabeled alpha-synuclein was made and this mixture was then incubated at 37°C with agitation (300 rpm) for 2 days, then a pause for 3 days, then 1 day of agitation, then 1 day pause, then 4 days of agitation. After that the fibrils were harvested at kept at -20°C until use. When fibrils were used in cellular assays, they were always sonicated at 5 min, setting 5.50% cycle, with horn probe sonicator, immediately prior to addition.

Antibody-mediated inhibition of accumulation in SK-mel5 cells

The human melanoma cell line SK-mel5 (ATCC, HTB-70) was grown in accordance with the ATCC-guidelines. Cells were plated at a density of 3000 cells per well in Falcon BD 96-well plates and left to adhere overnight. Atto488-labelled alpha-synuclein fibrils were added to the cells (0.01 mg/ml) together with m2E6 antibody (0.01 mg/ml) and alpha-synuclein peptides 113-125 or 126-140 (0.01 mg/ml). After 24 hours of incubation, the cells were washed twice in PBS and fixed by 4 % paraformaldehyde. The cells were then stained with Hoechst and read in Cellomics ArrayScan. Nuclei were detected in one channel and defined the number of valid objects. Atto488-labelled fibrils were detected in another channel in a pre-defined ring-formed area surrounding the nucleus, thus representing the cytoplasm of the cells. The percent of the cells containing alpha-synuclein spots was quantified. The result shows that in cells not given fibrils, there was only a very low background of spot-containing cells (background were probably due to autofluorescence) Fig 7C. In the cells given fibrils only, 75 % of the cells had accumulated intracellular spots. In the cells co-incubated with fibrils and m2E6 antibody, there were only around 30% spot-positive cells. When the cells were co-incubated with fibrils, m2E6 and the 126-140 peptide, there were around 60% positive cells, thus the peptide significantly inhibited the effect of m2E6. Co-incubation of the 113-120 peptide with fibrils and 2E6 did not change the effect of m2E6. Incubation of fibrils together with either of the peptides 113-120 or 126-140 had no effect on the accumulation of fibrils in the cells. Thus, the m2E6 binds to the alpha-synuclein fibrils in solution and inhibits their accumulation in the cells.

Treatment with increasing doses of 2E6-HLD1 showed a dose-dependent reduction in the percentage of cells with spots. Cells treated with irrelevant control antibody (B12) showed no effect.

5

B. Synuclein ELISA

Antibody-positive fusions were analysed for binding using an antigen-specific ELISA assay. Corning 96 well high binding plates were coated with 100 ng of aggregated synuclein. Wells were blocked using 5% milk in PBS for 1 hour (hr) at room temperature (RT). Plates were washed 3 times using PBS+1% Tween 20. One hundred microliters of hybridoma supernatant were added to each well and plates were incubated at RT. Subsequently, HRP-conjugated goat anti-mouse IgG (H&L chain-specific or γ -chain specific) secondary was added to each well to detect the presence of bound anti-synuclein antibody. For quantification substrate, One component TMB, was added and plates were measured at OD620.

C. Determining the DNA sequence of antibody HC and LC variable domains

Four anti-alpha synuclein positive hybridomas were selected and mRNA was extracted from cell pellets. cDNAs from each mRNA prep were generated by reverse-transcriptase using oligo(dT) primers. Subsequently, PCR reactions were performed using variable domain primers to amplify both the VH and VL regions of the HC and LC genes. Amplified DNA was separated on an agarose gel and both the VH and VL products were isolated, purified from the gel, cloned into pCR2.1 (Invitrogen) and transformed into TOP10 cells. A minimum of 6 positive colonies were selected and analysed by DNA sequencing to determine the sequence of the VH and VL regions.

Example 7: Antibody Engineering

30 *Expression of Monoclonal Antibodies*

Cultures of hybridoma clones were expanded and mouse monoclonal antibodies were purified from the cultured supernatants using protein G chromatography.

Recombinant mouse, human and chimeric antibodies were produced using transient co-transfection of heavy and light chain genes into HEK293 cells, expansion of the cultures, harvesting the supernatants and purification by protein chromatography. Instances where there was repeated need for gram quantities of antibodies stable cell lines were created in CHO cells. These stable cell lines could be expanded as needed and antibody purification was performed as before.

Cloning of recombinant antibodies

Recombinant monoclonal antibodies were generated by gene synthesis of the heavy and light chain genes (Geneart A/G). Synthesized genes were subsequently cloned into standard expression vectors (e.g. pcDNA3.1) for expression in mammalian cell culture.

Humanization

Humanization of m2E6 was carried out by structure based CDR grafting. The amino acid sequences of the 2E6 VL and VH domains were screened for homology against all human antibody VL and VH framework amino acid sequences found in the PDB and IMGT databases. Structural modeling was performed on the m2E6 Fv region using 20SL antibody from the PDB database. The 20SL amino acid sequences are 82.7% and 83.2% homologous to the 2E6 VH and VL domains, respectively. Importantly the structure for 20SL was determined at a resolution of 2.1Å. Structural alignment of the 2E6 humanized framework with 20SL enabled determination of important residues in the framework regions that could potentially influence folding or local structure via steric hindrance or steric force. Theoretical antibody structural modeling of the humanized antibody was employed to instruct on the potential importance of maintaining specific residues as the original mouse amino acid in the humanized version of 2E6 in order to maintain binding specificity and affinity. The structural modeling was employed to optimize the activity of humanized 2E6.

30

Humanization of the 2E6 VH region was performed by grafting the VH CDRs onto the framework of the human germline gene, IGHV1-46*01 (69% homology). There are 23 amino acid differences between the mouse 2E6 and the selected human

framework regions. Structural modeling identified 7 amino acid positions where the change to the human residue had the potential to negatively impact the activity of 2E6. These residues were back-mutated to the original mouse amino acids. Three different versions of the humanized heavy chain were produced. Humanized

5 HLD-1 contains all 7 back mutations, M37V, I48M, A68V, L70M, V72R, K74T, A79V, HLD-2 contains I48M, A68V, L70M, V72R, K74T, A79V, and HLD-3 contains M37V, I48M, L70M, V72R, K74T, A79V.

Humanization of the 2E6 VL region was performed by grafting the VL CDRs onto the framework of the human germline gene, IGKV3-11*01 (64% homology). There are 26 amino acid differences between the mouse 2E6 and the selected human framework regions. Structural modeling identified 4 amino acid positions, R45L, W46L, V57I, Y70F, where the change to the human residue had the potential to negatively impact the activity of 2E6. For HLD-1, HLD-2 and HLD-3 all 4 residues

15 were back-mutated to the original mouse amino acids.

HLD-1, HLD-2 and HLD-3 were expressed transiently in HEK 293 cells. Antibodies were purified from cultured supernatants and subsequently analyzed for binding to synuclein by SPR (Biacore 3000) using the synuclein ligand format (Table 5).

20

Table 5: Kinetic binding analysis of different humanized 2E6 clones and chimeric 2E6

	ka {1/Ms}	kd {1/s}	KA {1/M}	KD {nM}	Chi2	KD improvement
Ch2E6	6,29E+04	2,65E-04	2,38E+08	4,21E-09	3,57	1
HLD1	1,23E+05	2,12E-04	5,81E+08	1,72E-09	4,56	2
HLD2	5,80E+04	2,85E-04	2,04E+08	4,91E-09	4,34	1
HLD3	3 4,89E+04	2,60E-04	1,88E+08	5,32E-09	2,79	1

Affinity maturation of HLD1 was done by randomized mutations in the light chain CDR3 by codon based degenerated PCR primers, and similarly randomized mutations in the heavy chain CDR3 by codon based degenerated PCR primers and using in vitro evolution with error-prone PCR. Antibodies were purified from cultured supernatants and subsequently analyzed for binding to synuclein by SPR (Biacore 3000) using IgGs captured using anti human IgG Ab immobilized on the CM5 chip (Table 6).

Table 6: Kinetic binding analysis of different affinity matured versions of humanized 2E6 clone HLD1 – after first round of affinity maturation

	ka (1/Ms)	kd (1/s)	KA (1/M)	KD (M)	Chi2	KD improvement
Ch2E6	2.45E+04	1.39E-03	1.76E+07	5.67E-08	0.22	1
HLD1	4.16E+04	9.44E-04	4.40E+07	2.27E-08	0.164	2.5
L3-11	1.45E+05	3.16E-04	4.60E+08	2.18E-09	0.285	26
7A10	5.17E+04	2.85E-04	1.81E+08	5.52E-09	0.297	10.3
9C12	4.95E+04	2.78E-04	1.78E+08	5.62E-09	0.631	10
8D9	7.41E+04	4.83E-04	1.53E+08	6.52E-09	0.301	8.7
7C4	1.23E+05	9.97E-04	1.23E+08	8.12E-09	1.04	7

After first round of affinity maturation we constructed 4 mutations (A, B, C, D): A) combined the two mutations in heavy chain CDR2 (mutate KYNVNFKT to KYNVNIKT) and heavy chain CDR3 (mutate LGHYGNLYAMDY to LGHYGNLYAKDY); B) incorporated light chain CDR1 mutation (mutate SASSSVSYM to SASSSVSYIH) into the L3-11 light chain; C) incorporated light chain framework mutation (mutate PRRWIY to PRRLIY, immediately upstream CDR2) into the L3-11 light chain; and D) incorporated light chain CDR1 mutation (mutate SASSSVSYM to SASSSVSYIH) and light chain framework mutation (mutate PRRWIY to PRRLIY) into the L3-11 light chain. Based on the Biacore data

and antibody sequence, we tested co-expression of light chain and heavy chain with various combinations:

1. L3-11 light chain + 9C12 heavy chain
2. L3-11 light chain + 8D9 heavy chain
- 5 3. 7A10 light chain + 9C12 heavy chain
4. L3-11 light chain + A
5. 7A10 light chain + A
9. B + 9C12 heavy chain
10. C + 9C12 heavy chain
- 10 11. D + 9C12 heavy chain
12. B + 8D9 heavy chain
13. C + 8D9 heavy chain
14. D + 8D9 heavy chain
15. B + heavy chain
- 15 16. C + heavy chain
17. D + heavy chain

Antibodies were purified from cultured supernatants and subsequently analyzed for binding to synuclein by SPR (Biacore 3000) using IgGs captured using anti human IgG Ab immobilized on the CM5 chip (Table 7).

20

Table 7: Kinetic binding analysis of different affinity matured versions of humanized 2E6 clone HLD1 – after combination of mutations

	k_a (1/Ms)	k_d (1/s)	K_A (1/M)	KD (M)	Chi2	KD improvement
Ch2E6	2.45E+04	1.39E-03	1.76E+07	5.67E-08	0.22	1
HLD1	4.16E+04	9.44E-04	4.40E+07	2.27E-08	0.164	2,5
HLD1-14	1.35E+05	5.60E-05	2.42E+09	4.14E-10	0.03	137,0
HLD1-12	2.47E+05	1.12E-04	2.21E+09	4.51E-10	0.12	125,7
HLD1-13	1.46E+05	7.07E-05	2.07E+09	4.83E-10	0.11	117,4
HLD1-15	2.58E+05	1.25E-04	2.06E+09	4.85E-10	0.09	116,9
HLD1-9	2.60E+05	1.33E-04	1.94E+09	5.14E-10	0.06	110,3

HLD1-16	1.53E+05	8.97E-05	1.71E+09	5.85E-10	0.14	96,9
HLD1-2	2.38E+05	1.52E-04	1.57E+09	6.36E-10	0.06	89,2
HLD1-3	9.99E+04	1.26E-04	7.94E+08	1.26E-09	0.06	45,0
HLD1-5	9.29E+04	1.28E-04	7.27E+08	1.38E-09	0.03	41,1

Example 8

Frontal cortex from AD patients were homogenized in ice cold sterile PBS and homogenized by a knife homogenizer, sonicated using a Branson sonifier (5 pulses 0,9 seconds, output 2) and cleared at 3000 g, 5 min at
5 4C. Supernatants were collected and protein concentrations determined by BCA. Samples used to determine the level of alpha-synuclein aggregates contained from 2.1-4.8 ug/ μ l protein. Alpha-synuclein aggregates was measured using the commercial available alpha-synuclein aggregation assay from Cisbio (cat no 6FASYPEG). Alpha-synuclein phosphorylated on serine129 (synuclein-p129), a marker for Lewy bodies, was measured using the upcoming commercial available synuclein-p129 aggregation assay from Cisbio. The two assays were performed according to the manufacturers protocols using the respective buffers from each kit. Briefly, all 10% homogenates were serially diluted 1:12 and 1:72 in either of the lysis buffers and 10 μ l sample was mixed with 10 μ l of the respective antibody mix (5 μ l Tb-cryptate antibody and 5 μ l d2 conjugate) and incubated for 20 hours. Time-resolved FRET was measured on the Pherastar plate reader and signal-to-noise ratio was calculated and normalized to protein for each sample.

20

Fifty fresh frozen tissue samples of frontal cortex from AD patients were obtained from the Banner Sun Health Research Institute Brain and Body Donation program (BBDP), Sun city, Arizona, US. 25 samples from patients with mid-stage AD (Braak stage III/IV) and 25 samples from patients
25 with late-stage AD (Braak stage V/VI) was obtained. None of the samples were reported to contain immunohistochemically evidence of alpha-synuclein pathology.

Results are shown in figure 9. Alpha-synuclein aggregates can be measured in all 50 AD cases. In figure 9A raw data from 12- and 72- fold dilutions of 10% brain homogenates (W/V) show a concentration dependent signal intensity. Two samples from patients with Dementia with Lewy bodies (DLB) were included as positive controls (red lines in figure 9A). In figure 9B the data from the 1:12 dilution is normalized to total protein in the samples showing the presence of alpha-synuclein aggregates in similar levels in both mid-stage AD (Braak stage III/IV) and late-stage AD (Braak stage V/VI).

10

Alpha-synuclein phosphorylated on serine 129 was not detected in any of the AD samples, whereas it was clearly present in the DLB samples (figure 9C). Alpha-synuclein phosphorylated on serine 129 is a marker for manifest Lewy body pathology and histological staining of post mortem brain with alpha-synuclein-serine129-phospho antibodies are routinely used to confirm the diagnosis of synucleopathies like Parkinson's disease and DLB. The absence of this marker in the 50 AD samples might indicate that alpha-synuclein aggregates are always present in AD brains – and that synuclein aggregates can be present without the presence of manifest Lewy body pathology.

20

Based on these findings in combination with the findings in figure 8, we hypothesize that any alpha-synuclein antibody that are capable of neutralizing alpha-synuclein aggregates (seeds) or by other means prevent alpha-synuclein aggregates in entering neurons or glia cells and facilitate aggregation of Tau, will have a therapeutic potential to treat tauopathies.

25

30

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15

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 35 40 45

20

Ser Ala Ile Arg Ser Asn Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val
 50 55 60

25

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

30

Ala Lys Asn Trp Ala Pro Phe Asp Ser Trp Gly Gln Gly Thr Leu Val
 100 105 110

35 Thr

<210> 8
 <211> 108
 5 <212> PRT
 <213> Artificial

 <220>
 <223> GM 37 Light Chain
 10
 <400> 8

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

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

5 <210> 9
 <211> 6
 <212> PRT
 <213> Artificial

10 <220>
 <223> Epitope 112-117
 <400> 9

15 Ile Leu Glu Asp Met Pro
 1 5

<210> 10
 20 <211> 140
 <212> PRT
 <213> Artificial

<220>
 25 <223> Alpha-synuclein
 <400> 10

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 30 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30
 35

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

5 Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 10 65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95
 15

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110

20
 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125

25 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

30 <210> 11
 <211> 165
 <212> PRT
 <213> Artificial

<220>
 35 <223> A-Syn-AAKK-BAP

<400> 11

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15
 5

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

10 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

15 Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 20 65 70 75 80

Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys
 85 90 95

25 Lys Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly
 100 105 110

30 Met Val Asn Asn Thr Asp Ile Pro Val Asp Pro Glu Asn Glu Ala Tyr
 115 120 125

35 Glu Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala Gly
 130 135 140

Ser Ala Gly Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys
 145 150 155 160
 5

Ile Glu Trp His Glu
 165

10

<210> 12
 <211> 165
 <212> PRT
 <213> Artificial

15

<220>
 <223> A-Syn-BAAK-BAP

<400> 12

20

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

25

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 30 35 40 45

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

35

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

5 Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

10 Lys Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly
 100 105 110

15 Met Val Asn Asn Thr Asp Ile Pro Val Asp Pro Glu Asn Glu Ala Tyr
 115 120 125

Glu Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala Gly
 130 135 140

20 Ser Ala Gly Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys
 145 150 155 160

25 Ile Glu Trp His Glu
 165

30 <210> 13
 <211> 162
 <212> PRT
 <213> Artificial

<220>
 35 <223> A-Syn-BBAA-BAP

<400> 13

Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15
 5

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30

10 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Arg Glu Gly Val
 35 40 45

15 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60

His Leu Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 20 65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

25

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110

30 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125

35 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala Gly Ser Ala Gly
 130 135 140

Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp
 145 150 155 160
 5
 His Glu
 10
 <210> 14
 <211> 165
 <212> PRT
 <213> Artificial
 15
 <220>
 <223> A-Syn-BBKK-BAP
 <400> 14
 20
 Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15
 25 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30
 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Arg Glu Gly Val
 30 35 40 45
 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60
 35

His Leu Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

5 Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys
 85 90 95

10 Lys Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly
 100 105 110

15 Met Val Asn Asn Thr Asp Ile Pro Val Asp Pro Glu Asn Glu Ala Tyr
 115 120 125

20 Glu Met Pro Pro Glu Glu Glu Tyr Gln Asp Tyr Glu Pro Glu Ala Gly
 130 135 140

25 Ser Ala Gly Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys
 145 150 155 160

25 Ile Glu Trp His Glu
 165

30 <210> 15
 <211> 141
 <212> PRT
 <213> Artificial

35 <220>
 <223> A-Syn-120-140_Del-BAP

<400> 15

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15
 5

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30

10
 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

15 Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 20 65 70 75 80

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

25
 Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110

30
 Leu Glu Asp Met Pro Val Asp Gly Ser Ala Gly Gly Ser Gly Gly Leu
 115 120 125

35 Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
 130 135 140

<210> 16
 <211> 131
 5 <212> PRT
 <213> Artificial

 <220>
 <223> alpha-synuclein amino acids 1-119
 10
 <400> 16

 Met Ala His His His His His His Ile Glu Gly Arg Met Asp Val Phe
 1 5 10 15
 15
 Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val Ala Ala Ala Glu
 20 25 30
 20
 Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr Lys Glu Gly
 35 40 45
 25 Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val Val His Gly Val
 50 55 60
 Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr Asn Val Gly Gly
 30 65 70 75 80
 Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr Val Glu Gly
 85 90 95
 35

Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys Lys Asp Gln Leu
100 105 110

5 Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile Leu Glu Asp Met
115 120 125

Pro Val Asp
10 130

<210> 17
<211> 106
15 <212> PRT
<213> artificial

<220>
<223> kappa (LC constant region)
20 <400> 17

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15
25

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
20 25 30

30 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
35 40 45

35 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
50 55 60

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 65 70 75 80
 5

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 85 90 95

10

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105

15 <210> 18
 <211> 329
 <212> PRT
 <213> Artificial

20 <220>
 <223> IgG1 (HC Constant region)
 <400> 18

25 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 30 20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

35

Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
50 55 60

5 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
10 85 90 95

Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
100 105 110

15

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
115 120 125

20

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
130 135 140

25 Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
30 165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
180 185 190

35

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 195 200 205

5 Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu
 10 225 230 235 240

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 245 250 255

15

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 260 265 270

20

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 275 280 285

25 Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 30 305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly
 325

35

<210> 19
<211> 4
<212> PRT
<213> Artificial

5
<220>
<223> GM285 epitope 112-115

<400> 19

10
Ile Leu Glu Asp
1

15 <210> 20
<211> 13
<212> PRT
<213> Artificial

20 <220>
<223> GM285 CDR1 Heavy Chain

<400> 20

25 Ala Ala Ser Gly Phe Thr Phe Ser Arg Phe Thr Met Thr
1 5 10

<210> 21

30 <211> 17
<212> PRT
<213> Artificial

<220>

35 <223> GM285 CDR2 Heavy Chain

<400> 21

Ala Ile Ser Gly Ser Gly Gly Gly Thr Ser Tyr Ala Asp Ser Val Lys
1 5 10 15

5

Gly

10

<210> 22

<211> 9

<212> PRT

<213> Artificial

15

<220>

<223> GM285 CDR3 Heavy Chain

<400> 22

20

Ala Lys Asn Trp Ala Pro Phe Asp Tyr
1 5

25

<210> 23

<211> 12

<212> PRT

<213> Artificial

30

<220>

<223> GM285 CDR1 Light Chain

<400> 23

35

Arg Ala Ser Gln Ser Val Ser Arg Ser Tyr Leu Ala
1 5 10

<210> 24
<211> 7
5 <212> PRT
<213> Artificial

<220>
<223> GM285 CDR2 Light Chain
10
<400> 24

Gly Ala Ser Ser Arg Ala Thr
1 5
15

<210> 25
<211> 9
<212> PRT
20 <213> Artificial

<220>
<223> GM285 CDR3 Light Chain

25 <400> 25

Gln Gln Tyr Gly Ser Ser Pro Trp Thr
1 5

30
<210> 26
<211> 113
<212> PRT
<213> Artificial
35
<220>

<223> GM285 VH

<400> 26

5 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Arg Phe
10 20 25 30

Thr Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
15 35 40 45

Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Ser Tyr Ala Asp Ser Val
50 55 60

20 Lys Gly Arg Leu Thr Val Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr
65 70 75 80

25 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

Ala Lys Asn Trp Ala Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
30 100 105 110

Thr

35

<210> 27
 <211> 108
 <212> PRT
 <213> Artificial

5

<220>
 <223> GM285 VL

<400> 27

10

Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

15

Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
 20 25 30

20

Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45

25

Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60

Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Val Ser Arg Leu Glu
 65 70 75 80

30

Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95

35

Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

<210> 28
 <211> 329
 5 <212> PRT
 <213> Artificial

 <220>
 <223> GM285 IgG1 constant region
 10
 <400> 28

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

118

Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
245 250 255

5 Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
10 275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
290 295 300

15 Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
305 310 315 320

20 Gln Lys Ser Leu Ser Leu Ser Pro Gly
325

25 <210> 29
<211> 106
<212> PRT
<213> Artificial

30 <220>
<223> GM285 Kappa chain

<400> 29

35 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
1 5 10 15

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 20 25 30
 5

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 35 40 45

10

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 50 55 60

15

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 65 70 75 80

20

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 85 90 95

25

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105

30

<210> 30
 <211> 113
 <212> PRT
 <213> Artificial

<220>
 <223> GM37 Variant 1 heavy chain

35 <400> 30

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
 1 5 10 15

5 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 10 35 40 45

Ser Ala Ile Arg Ser Ser Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val
 50 55 60

15 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Thr Leu Tyr
 65 70 75 80

20 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

25 Ala Lys Asn Trp Ala Pro Phe Asp Ser Trp Gly Gln Gly Thr Leu Val
 100 105 110

30 Thr

35 <210> 31
 <211> 113
 <212> PRT
 <213> Artificial

<220>

<223> GM 37 variant 2 heavy chain

5 <400> 31

Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
 1 5 10 15

10

Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30

15

Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45

20

Ser Ala Ile Arg Ser Gln Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val
 50 55 60

25

Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Gln Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

30

Ala Lys Asn Trp Ala Pro Phe Asp Ser Trp Gly Gln Gly Thr Leu Val
 100 105 110

35 Thr

<210> 32
 <211> 113
 5 <212> PRT
 <213> Artificial

 <220>
 <223> GM 37 variant 3 heavy chain
 10
 <400> 32

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

5 Thr

Ala Ile Arg Ser Ser Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
20 1 5 10 15

25

	<210>	34
	<211>	17
	<212>	PRT
30	<213>	Artificial
	<220>	
	<223>	GM37 variant 2 CDR 2 heavy chain
35	<400>	34

Ala Ile Arg Ser Gln Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
 1 5 10 15

5 Gly

10 <210> 35
 <211> 17
 <212> PRT
 <213> Artificial

15 <220>
 <223> GM37 variant 3 CDR 2 heavy chain

<400> 35

Ala Ile Arg Ser His Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
 20 1 5 10 15

Gly

25

30 <210> 36
 <211> 5
 <212> PRT
 <213> Artificial

<220>
 <223> 9E4 binding epitope

35 <400> 36

Asn Glu Ala Tyr Glu
 1 5

5 <210> 37
 <211> 134
 <212> PRT
 <213> Artificial

10 <220>
 <223> HUMAN Beta-synuclein
 <400> 37

15 Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Arg Glu Gly Val
 35 40 45

25 Val Gln Gly Val Ala Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60

30 His Leu Gly Gly Ala Val Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80

35 Thr Gly Leu Val Lys Arg Glu Glu Phe Pro Thr Asp Leu Lys Pro Glu
 85 90 95

Glu Val Ala Gln Glu Ala Ala Glu Glu Pro Leu Ile Glu Pro Leu Met
100 105 110

5

Glu Pro Glu Gly Glu Ser Tyr Glu Asp Pro Pro Gln Glu Glu Tyr Gln
115 120 125

10

Glu Tyr Glu Pro Glu Ala
130

15 <210> 38
<211> 127
<212> PRT
<213> Artificial

20 <220>
<223> HUMAN Gamma-synuclein

<400> 38

25 Met Asp Val Phe Lys Lys Gly Phe Ser Ile Ala Lys Glu Gly Val Val
1 5 10 15

Gly Ala Val Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
30 20 25 30

Thr Lys Glu Gly Val Met Tyr Val Gly Ala Lys Thr Lys Glu Asn Val
35 40 45

35

Val Gln Ser Val Thr Ser Val Ala Glu Lys Thr Lys Glu Gln Ala Asn
50 55 60

5 Ala Val Ser Glu Ala Val Val Ser Ser Val Asn Thr Val Ala Thr Lys
65 70 75 80

Thr Val Glu Glu Ala Glu Asn Ile Ala Val Thr Ser Gly Val Val Arg
10 85 90 95

Lys Glu Asp Leu Arg Pro Ser Ala Pro Gln Gln Glu Gly Glu Ala Ser
100 105 110

15

Lys Glu Lys Glu Glu Val Ala Glu Glu Ala Gln Ser Gly Gly Asp
115 120 125

20

<210> 39
<211> 140
<212> PRT
<213> Artificial

25

<220>
<223> alpha-synuclein ortholog for Cynomolgus monkey

<400> 39

30

Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
1 5 10 15

35 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45
 5

Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60
 10

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80
 15

Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Ile Lys
 85 90 95
 20

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110
 25

Leu Gln Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125
 30

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140
 35

<210> 40
 <211> 140
 <212> PRT
 <213> Artificial
 <220>

<223> alpha-synuclein ortholog for Rat

<400> 40

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5  Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
   1              5              10              15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
10              20              25              30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
              35              40              45
15

Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
              50              55              60

20

Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
65              70              75              80

25  Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
              85              90              95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
30              100             105             110

Leu Glu Asp Met Pro Val Asp Pro Ser Ser Glu Ala Tyr Glu Met Pro
              115             120             125
35

```

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

5 <210> 41
 <211> 140
 <212> PRT
 <213> Artificial

10 <220>
 <223> alpha-synuclein ortholog for Mouse
 <400> 41

15 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15

Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 20 25 30

Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45

25 Val His Gly Val Thr Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60

30 Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80

35 Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95

Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110
 5

Leu Glu Asp Met Pro Val Asp Pro Gly Ser Glu Ala Tyr Glu Met Pro
 115 120 125

10

Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

15 <210> 42
 <211> 446
 <212> PRT
 <213> artificial

20 <220>
 <223> 9E4 HC
 <400> 42

25 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

30 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr
 20 25 30

Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 35

Ala Ser Ile Ser Ser Gly Gly Gly Ser Thr Tyr Tyr Pro Asp Asn Val
50 55 60

5 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65 70 75 80

Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
10 85 90 95

Ala Arg Gly Gly Ala Gly Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val
100 105 110

15 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
115 120 125

20 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
130 135 140

25 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
145 150 155 160

Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
30 165 170 175

Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
180 185 190

35

Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 195 200 205

5 Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 210 215 220

Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 10 225 230 235 240

Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 245 250 255

15

Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 260 265 270

20

Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 275 280 285

25 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
 290 295 300

Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
 30 305 310 315 320

Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 325 330 335

35

Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 340 345 350

5 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 355 360 365

10 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 370 375 380

15 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 385 390 395 400

Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
 405 410 415

20 Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala Leu His
 420 425 430

25 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 435 440 445

30 <210> 43
 <211> 220
 <212> PRT
 <213> artificial

35 <220>
 <223> 9E4 LC

<400> 43

Asp Ile Gln Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1 5 10 15
 5

Asp Arg Val Thr Ile Thr Cys Lys Ser Ile Gln Thr Leu Leu Tyr Ser
 20 25 30
 10

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys
 35 40 45
 15

Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ile Arg Lys Ser Gly Val
 50 55 60
 20

Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80
 25

Ile Ser Ser Leu Gln Pro Glu Asp Leu Ala Thr Tyr Tyr Cys Gln Gln
 85 90 95
 30

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 100 105 110
 35

Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125
 35

Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140

Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160
 5

Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 165 170 175

10

Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 180 185 190

15

Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 195 200 205

20

Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215 220

25

<210> 44
 <211> 10
 <212> PRT
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<220>
 <223> 9E4 CDR1 Heavy Chain

35

Gly Phe Thr Phe Ser Asn Tyr Gly Met Ser
 1 5 10

<210> 45
 <211> 17
 <212> PRT
 <213> artificial
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 <220>
 <223> 9E4 CDR2 Heavy Chain

 <400> 45
 10
 Ser Ile Ser Ser Gly Gly Gly Ser Thr Tyr Tyr Pro Asp Asn Val Lys
 1 5 10 15

 15 Gly

 <210> 46
 20 <211> 7
 <212> PRT
 <213> artificial

 <220>
 25 <223> 9E4 CDR3 Heavy Chain

 <400> 46

 Gly Gly Ala Gly Ile Asp Tyr
 30 1 5

 <210> 47
 <211> 17
 35 <212> PRT
 <213> artificial

<220>
<223> 9E4 CDR1 Light Chain

5 <400> 47

Lys Ser Ile Gln Thr Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu
1 5 10 15

10
Ala

15 <210> 48
<211> 7
<212> PRT
<213> artificial

20 <220>
<223> 9E4 CDR2 Light Chain

<400> 48

25 Trp Ala Ser Ile Arg Lys Ser
1 5

<210> 49

30 <211> 9
<212> PRT
<213> artificial

<220>

35 <223> 9E4 CDR3 Light Chain

<400> 49

 Gln Gln Tyr Tyr Ser Tyr Pro Leu Thr
 1 5
 5

 <210> 50
 <211> 13
 <212> PRT
 10 <213> artificial

 <220>
 <223> GM63 Epitope 126-138

 15 <400> 50

 Glu Met Pro Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro
 1 5 10

 20
 <210> 51
 <211> 13
 <212> PRT
 <213> artificial
 25
 <220>
 <223> GM63 CDR1 Heavy Chain

 <400> 51
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 Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr Gly Ile Ile
 1 5 10

 35 <210> 52
 <211> 10

<212> PRT
<213> artificial

<220>
5 <223> GM63 CDR2 Heavy Chain

<400> 52

Trp Ile Ser Ala Tyr Asn Gly Lys Thr Asn
10 1 5 10

<210> 53
<211> 10
15 <212> PRT
<213> artificial

<220>
<223> GM63 CDR3 Heavy Chain
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<400> 53

Thr Arg Ala His Trp Gly Arg Phe Asp Tyr
1 5 10
25

<210> 54
<211> 11
<212> PRT
30 <213> artificial

<220>
<223> GM63 CDR1 Light Chain

35 <400> 54

Arg Ala Ser Gln Gly Ile Ser Ser Ala Leu Ala
1 5 10

5 <210> 55
<211> 8
<212> PRT
<213> artificial

10 <220>
<223> GM63 CDR2 Light Chain

<400> 55

15 Tyr Asp Ala Ser Ser Leu Glu Ser
1 5

<210> 56
20 <211> 9
<212> PRT
<213> artificial

<220>
25 <223> GM63 CDR3 Light Chain

<400> 56

Gln Gln Phe Lys Ser Tyr Pro Arg Thr
30 1 5

<210> 57
<211> 114
35 <212> PRT
<213> artificial

<220>

<223> GM63 VH

5 <400> 57

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

10

Ser Val Ile Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
 20 25 30

15

Gly Ile Ile Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met
 35 40 45

20

Gly Trp Ile Ser Ala Tyr Asn Gly Lys Thr Asn Tyr Ala Gln Asn Leu
 50 55 60

25

Gln Gly Arg Val Thr Met Thr Thr Asp Thr Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Arg Ser Leu Arg Ser Asp Asp Thr Ala Met Tyr Tyr Cys
 85 90 95

30

Thr Arg Ala His Trp Gly Arg Phe Asp Tyr Trp Gly Gln Gly Thr Leu
 100 105 110

35 Val Thr

<210> 58
 <211> 107
 5 <212> PRT
 <213> artificial

 <220>
 <223> GM63 VL
 10
 <400> 58

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

Thr Leu Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105

5 <210> 59
 <211> 701
 <212> PRT
 <213> artificial

10 <220>
 <223> GM63 Heavy CHain Constant Domain
 <400> 59

15 Gly Cys Cys Thr Cys Cys Ala Cys Cys Ala Ala Gly Gly Gly Cys Cys
 1 5 10 15

Cys Ala Thr Cys Gly Gly Thr Cys Thr Thr Cys Cys Cys Ala Cys Thr
 20 20 25 30

Gly Gly Cys Gly Cys Cys Cys Thr Cys Cys Thr Cys Cys Ala Ala Gly
 35 40 45

25

Ala Gly Cys Ala Cys Cys Ala Gly Cys Gly Gly Cys Gly Gly Cys Ala
 50 55 60

30

Cys Ala Gly Cys Cys Gly Cys Cys Cys Thr Gly Gly Gly Cys Thr Gly
 65 70 75 80

35 Cys Cys Thr Gly Gly Thr Gly Ala Ala Gly Gly Ala Cys Thr Ala Cys
 85 90 95

Thr Thr Cys Cys Cys Cys Gly Ala Gly Cys Cys Ala Gly Thr Gly Ala
 100 105 110
 5

Cys Cys Gly Thr Gly Thr Cys Cys Thr Gly Gly Ala Ala Cys Thr Cys
 115 120 125

10

Thr Gly Gly Cys Gly Cys Cys Cys Thr Gly Ala Cys Cys Thr Cys Cys
 130 135 140

15

Gly Gly Cys Gly Thr Gly Cys Ala Cys Ala Cys Cys Thr Thr Cys Cys
 145 150 155 160

20

Cys Cys Gly Cys Cys Gly Thr Gly Cys Thr Gly Cys Ala Gly Ala Gly
 165 170 175

25

Cys Ala Gly Cys Gly Gly Cys Cys Thr Gly Thr Ala Cys Ala Gly Cys
 180 185 190

30

Cys Thr Gly Ala Gly Cys Ala Gly Cys Gly Thr Gly Gly Thr Gly Ala
 195 200 205

35

Cys Cys Gly Thr Gly Gly Gly Cys Ala Cys Cys Cys Ala Gly Ala Cys Cys
 210 215 220

225 230 235 240

Thr Ala Cys Ala Thr Cys Thr Gly Cys Ala Ala Cys Gly Thr Gly Ala
 245 250 255
 5

Ala Cys Cys Ala Cys Ala Ala Gly Cys Cys Cys Ala Gly Cys Ala Ala
 260 265 270

10

Cys Ala Cys Cys Ala Ala Gly Gly Thr Gly Gly Ala Cys Ala Ala Gly
 275 280 285

15

Ala Gly Ala Gly Thr Gly Gly Ala Gly Cys Cys Cys Ala Ala Gly Ala
 290 295 300

Gly Cys Thr Gly Cys Gly Ala Cys Ala Ala Gly Ala Cys Cys Cys Ala
 20 305 310 315 320

Cys Ala Cys Cys Thr Gly Cys Cys Cys Cys Cys Cys Cys Thr Gly Cys
 325 330 335

25

Cys Cys Ala Gly Cys Cys Cys Cys Ala Gly Ala Gly Cys Thr Gly Cys
 340 345 350

30

Thr Gly Gly Gly Cys Gly Gly Ala Cys Cys Cys Ala Gly Cys Gly Thr
 355 360 365

35

Gly Thr Thr Cys Cys Thr Gly Thr Thr Cys Cys Cys Cys Cys Cys Cys
 370 375 380

Ala Ala Gly Cys Cys Cys Ala Ala Gly Gly Ala Cys Ala Cys Cys Cys
 385 390 395 400
 5

Thr Gly Ala Thr Gly Ala Thr Cys Ala Gly Cys Ala Gly Gly Ala Cys
 405 410 415

10
 Cys Cys Cys Cys Gly Ala Gly Gly Thr Gly Ala Cys Cys Thr Gly Cys
 420 425 430

15 Gly Thr Gly Gly Thr Gly Gly Thr Gly Gly Ala Cys Gly Thr Gly Ala
 435 440 445

Gly Cys Cys Ala Cys Gly Ala Gly Gly Ala Cys Cys Cys Ala Gly Ala
 20 450 455 460

Gly Gly Thr Gly Ala Ala Gly Thr Thr Cys Ala Ala Cys Thr Gly Gly
 465 470 475 480
 25

Thr Ala Cys Gly Thr Gly Gly Ala Cys Gly Gly Cys Gly Thr Gly Gly
 485 490 495

30
 Ala Gly Gly Thr Gly Cys Ala Cys Ala Ala Cys Gly Cys Cys Ala Ala
 500 505 510

35 Gly Ala Cys Cys Ala Ala Gly Cys Cys Cys Ala Gly Ala Gly Ala Gly
 515 520 525

Gly Ala Gly Cys Ala Gly Thr Ala Cys Ala Ala Cys Ala Gly Cys Ala
 530 535 540
 5

Cys Cys Thr Ala Cys Ala Gly Gly Gly Thr Gly Gly Thr Gly Thr Cys
 545 550 555 560

10
 Cys Gly Thr Gly Cys Thr Gly Ala Cys Cys Gly Thr Gly Cys Thr Gly
 565 570 575

15 Cys Ala Cys Cys Ala Gly Gly Ala Cys Thr Gly Gly Cys Thr Gly Ala
 580 585 590

20 Ala Cys Gly Gly Cys Ala Ala Gly Gly Ala Ala Thr Ala Cys Ala Ala
 595 600 605

Gly Thr Gly Cys Ala Ala Gly Gly Thr Cys Thr Cys Cys Ala Ala Cys
 610 615 620
 25

Ala Ala Gly Gly Cys Cys Cys Thr Gly Cys Cys Ala Gly Cys Cys Cys
 625 630 635 640

30
 Cys Cys Ala Thr Cys Gly Ala Ala Ala Ala Gly Ala Cys Cys Ala Thr
 645 650 655

35 Cys Ala Gly Cys Ala Ala Gly Gly Cys Cys Ala Ala Gly Gly Gly Cys
 660 665 670

Cys Ala Gly Cys Cys Ala Cys Gly Gly Gly Ala Gly Cys Cys Cys Cys
 675 680 685
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Ala Gly Gly Thr Gly Thr Ala Cys Ala Cys Cys Cys Thr
 690 695 700

10

<210> 60
 <211> 323
 <212> PRT
 <213> artificial

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<220>
 <223> GM63 Kappa Light Chain Constant Domain
 <400> 60

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Gly Ala Ala Cys Thr Gly Thr Gly Gly Cys Thr Gly Cys Ala Cys Cys
 1 5 10 15

25

Ala Thr Cys Thr Gly Thr Cys Thr Thr Cys Ala Thr Cys Thr Thr Cys
 20 25 30

Cys Cys Gly Cys Cys Ala Thr Cys Thr Gly Ala Thr Gly Ala Gly Cys
 30 35 40 45

Ala Gly Thr Thr Gly Ala Ala Ala Thr Cys Thr Gly Gly Ala Ala Cys
 50 55 60

35

Thr Gly Cys Cys Thr Cys Ala Gly Thr Gly Gly Thr Gly Thr Gly Cys
 65 70 75 80

5 Cys Thr Gly Cys Thr Gly Ala Ala Cys Ala Ala Cys Thr Thr Cys Thr
 85 90 95

Ala Cys Cys Cys Cys Cys Gly Gly Gly Ala Gly Gly Cys Cys Ala Ala
 10 100 105 110

Gly Gly Thr Gly Cys Ala Gly Thr Gly Gly Ala Ala Gly Gly Thr Gly
 115 120 125
 15

Gly Ala Cys Ala Ala Cys Gly Cys Cys Cys Thr Gly Cys Ala Gly Ala
 130 135 140

20
 Gly Cys Gly Gly Cys Ala Ala Cys Ala Gly Cys Cys Ala Gly Gly Ala
 145 150 155 160

25 Gly Ala Gly Cys Gly Thr Cys Ala Cys Cys Gly Ala Gly Cys Ala Gly
 165 170 175

Gly Ala Cys Ala Gly Cys Ala Ala Gly Gly Ala Cys Thr Cys Cys Ala
 180 185 190
 30

Cys Cys Thr Ala Cys Ala Gly Cys Cys Thr Gly Ala Gly Cys Ala Gly
 195 200 205
 35

Cys Ala Cys Cys Cys Thr Gly Ala Cys Cys Cys Thr Gly Thr Cys Cys
 210 215 220

5 Ala Ala Gly Gly Cys Cys Gly Ala Cys Thr Ala Cys Gly Ala Gly Ala
 225 230 235 240

Ala Gly Cys Ala Cys Ala Ala Gly Gly Thr Gly Thr Ala Cys Gly Cys
 10 245 250 255

Cys Thr Gly Cys Gly Ala Gly Gly Thr Gly Ala Cys Cys Cys Ala Cys
 15 260 265 270

Cys Ala Gly Gly Gly Cys Cys Thr Gly Thr Cys Cys Ala Gly Cys Cys
 275 280 285

20 Cys Cys Gly Thr Gly Ala Cys Cys Ala Ala Gly Ala Gly Cys Thr Thr
 290 295 300

25 Cys Ala Ala Cys Ala Gly Gly Gly Gly Cys Gly Ala Gly Thr Gly Cys
 305 310 315 320

Thr Gly Ala
 30

<210> 61
 <211> 15
 35 <212> PRT
 <213> artificial

<220>
 <223> 9E4 Epitope 126-140

5 <400> 61

Glu Met Pro Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 1 5 10 15

10

<210> 62
 <211> 5
 <212> PRT
 <213> artificial

15

<220>
 <223> CDR1 VH

<400> 62

20

Ser Tyr Trp Met His
 1 5

25

<210> 63
 <211> 17
 <212> PRT
 <213> artificial

30

<220>
 <223> CDR2 VH

<400> 63

35 Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe Lys
 1 5 10 15

Thr

5

<210> 64
 <211> 12
 <212> PRT
 10 <213> artificial

<220>
 <223> CDR3 VH

15 <400> 64

Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr
 1 5 10

20

<210> 65
 <211> 10
 <212> PRT
 <213> artificial

25

<220>
 <223> CDR1 VL

<400> 65

30

Ser Ala Ser Ser Ser Val Ser Tyr Met His
 1 5 10

35 <210> 66
 <211> 7

<212> PRT
<213> artificial

<220>
5 <223> CDR2 VL

<400> 66

Asp Thr Ser Lys Leu Ala Ser
10 1 5

<210> 67
<211> 9
15 <212> PRT
<213> artificial

<220>
<223> CDR3 VL
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<400> 67

Gln Gln Trp Ser Ser Asn Pro Pro Thr
1 5
25

<210> 68
<211> 118
<212> PRT
30 <213> artificial

<220>
<223> m2E6 VH

35 <400> 68

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

5 Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Trp Met His Trp Met Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
 10 35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

15

Lys Thr Lys Ala Thr Leu Thr Val Asp Lys Pro Ser Ser Thr Ala Tyr
 65 70 75 80

20

Met His Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

25 Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Ser Val Thr
 30 115

<210> 69
 <211> 106
 35 <212> PRT
 <213> artificial

<220>

<223> m2E6 VL

5 <400> 69

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
 1 5 10 15

10

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30

15 His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
 35 40 45

20 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Thr Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Asp Thr Glu
 65 70 75 80

25

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

30

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
 100 105

35 <210> 70

<211> 118

<212> PRT

<213> artificial

<220>

5 <223> ch2E6 VH

<400> 70

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala
 10 1 5 10 15

Ser Val Lys Leu Ser Cys Thr Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30
 15

Trp Met His Trp Met Lys Gln Arg Pro Gly Arg Gly Leu Glu Trp Ile
 35 40 45
 20

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

Lys Thr Lys Ala Thr Leu Thr Val Asp Lys Pro Ser Ser Thr Ala Tyr
 65 70 75 80
 25

Met His Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95
 30

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
 100 105 110
 35

Gln Gly Thr Ser Val Thr
115

5 <210> 71
<211> 106
<212> PRT
<213> artificial

10 <220>
<223> ch2E6 VL

<400> 71

15 Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Met Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 20 25 30

His Trp Tyr Gln Gln Lys Ser Gly Thr Ser Pro Lys Arg Trp Ile Tyr
35 40 45

25 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Thr Arg Phe Ser Gly Ser
50 55 60

30 Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Ser Met Asp Thr Glu
65 70 75 80

35 Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95

Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

5

<210> 72
<211> 118
<212> PRT
10 <213> artificial

<220>
<223> 2E6-HLD1 VH

15 <400> 72

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

20

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

25 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
30 50 55 60

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80

35

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

5 Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
100 105 110

Gln Gly Thr Leu Val Thr
10 115

<210> 73
<211> 107
15 <212> PRT
<213> artificial

<220>
<223> 2E6-HLD1 VL
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<400> 73

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15
25

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
35 40 45

35 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

5 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95

10 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
100 105

15 <210> 74
<211> 118
<212> PRT
<213> Artificial

20 <220>
<223> 2E6-HLD2 VH

<400> 74

25 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
1 5 10 15

30 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

35 Trp Met His Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

5 Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 10 85 90 95

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
 100 105 110
 15

Gln Gly Thr Leu Val Thr
 115

20

<210> 75
 <211> 107
 <212> PRT
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25

<220>
 <223> 2E6-HLD2 VL

<400> 75

30

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

35 Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45
 5

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

10

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

15 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

20 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 76
 <211> 118
 25 <212> PRT
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<220>
 <223> 2E6-HLD 3 VH
 30

<400> 76

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 35

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

5 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 10 50 55 60

Lys Thr Arg Val Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

15 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

20 Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
 100 105 110

25 Gln Gly Thr Leu Val Thr
 115

30 <210> 77
 <211> 107
 <212> PRT
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<220>
 35 <223> 2E6-HLD 3 VL

<400> 77

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 5

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30

10

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45

15

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

20

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

25

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Asn
 85 90 95

30

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg
 100 105

<210> 78

<211> 16

<212> PRT

<213> Artificial

35

<220>

<223> D1.2 CDR 1 Light Chain

<400> 78

5 Arg Ser Ser Gln Ser Leu Val His Ser Asn Gly Asn Thr Tyr Leu His
 1 5 10 15

<210> 79

10 <211> 7
 <212> PRT
 <213> Artificial

<220>

15 <223> D1.2 CDR 2 Light Chain

<400> 79

Lys Val Ser Asn Arg Phe Ser

20 1 5

<210> 80

<211> 7

25 <212> PRT
 <213> Artificial

<220>

<223> D1.2 CDR 3 Light Chain

30 <400> 80

Ser Gln Ser Thr His Val Pro

35 1 5

<210> 81
 <211> 13
 <212> PRT
 <213> Artificial
 5
 <220>
 <223> D1.2 CDR 1 Heavy Chain

 <400> 81
 10
 Lys Ala Ser Gly Asn Thr Phe Thr Asp Tyr Glu Ile His
 1 5 10

 15 <210> 82
 <211> 17
 <212> PRT
 <213> Artificial

 20 <220>
 <223> D1.2 CDR 2 Heavy Chain

 <400> 82

 25 Ala Ile Asp Pro Glu Thr Gly Asn Thr Ala Tyr Asn Gln Lys Phe Lys
 1 5 10 15

 Gly
 30

 <210> 83
 <211> 6
 35 <212> PRT
 <213> Artificial

<220>
 <223> D1.2 CDR 3 Heavy Chain

 5 <400> 83

 Ser Arg Gly Phe Asp Tyr
 1 5

 10
 <210> 84
 <211> 219
 <212> PRT
 <213> Artificial
 15
 <220>
 <223> D1.2 Light Chain

 <400> 84
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 Asp Val Met Met Thr Gln Thr Pro Leu Ser Leu Pro Val Ser Leu Gly
 1 5 10 15

 25 Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val His Ser
 20 25 30

 Asn Gly Asn Thr Tyr Leu His Trp His Leu Gln Lys Pro Gly Gln Ser
 30 35 40 45

 Pro Lys Phe Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60
 35

	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Lys	Ile	
	65					70					75					80	
5	Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Val	Tyr	Phe	Cys	Ser	Gln	Ser	
					85					90					95		
	Thr	His	Val	Pro	Phe	Thr	Phe	Gly	Ser	Gly	Thr	Lys	Leu	Glu	Ile	Lys	
10				100					105					110			
	Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	
			115					120					125				
15																	
	Gln	Leu	Thr	Ser	Gly	Gly	Ala	Ser	Val	Val	Cys	Phe	Leu	Asn	Asn	Phe	
	130						135					140					
20																	
	Tyr	Pro	Lys	Asp	Ile	Asn	Val	Lys	Trp	Lys	Ile	Asp	Gly	Ser	Glu	Arg	
	145					150					155					160	
25	Gln	Asn	Gly	Val	Leu	Asn	Ser	Trp	Thr	Asp	Gln	Asp	Ser	Lys	Asp	Ser	
					165					170					175		
	Thr	Tyr	Ser	Met	Ser	Ser	Thr	Leu	Thr	Leu	Thr	Lys	Asp	Glu	Tyr	Glu	
30				180					185					190			
	Arg	His	Asn	Ser	Tyr	Thr	Cys	Glu	Ala	Thr	His	Lys	Thr	Ser	Thr	Ser	
			195					200					205				
35																	

Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys
 210 215

5 <210> 85
 <211> 451
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10 <220>
 <223> D1.2 Heavy Chain
 <400> 85

15 Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Val Arg Pro Gly Ala
 1 5 10 15

Ser Val Thr Leu Ser Cys Lys Ala Ser Gly Asn Thr Phe Thr Asp Tyr
 20 20 25 30

Glu Ile His Trp Val Lys Gln Thr Pro Val His Gly Leu Glu Trp Ile
 35 40 45

25

Gly Ala Ile Asp Pro Glu Thr Gly Asn Thr Ala Tyr Asn Gln Lys Phe
 50 55 60

30

Lys Gly Lys Ala Arg Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

35 Met Glu Leu Arg Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
 85 90 95

Thr Arg Ser Arg Gly Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu Thr
100 105 110

5

Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro
115 120 125

10

Gly Cys Gly Asp Thr Thr Gly Ser Ser Val Thr Leu Gly Cys Leu Val
130 135 140

15

Lys Gly Tyr Phe Pro Glu Ser Val Thr Val Thr Trp Asn Ser Gly Ser
145 150 155 160

20

Leu Ser Ser Ser Val His Thr Phe Pro Ala Leu Leu Gln Ser Gly Leu
165 170 175

25

Tyr Thr Met Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser
180 185 190

30

Gln Thr Val Thr Cys Ser Val Ala His Pro Ala Ser Ser Thr Thr Val
195 200 205

35

Asp Lys Lys Leu Glu Pro Ser Gly Pro Ile Ser Thr Ile Asn Pro Cys
210 215 220

35

Pro Pro Cys Lys Glu Cys His Lys Cys Pro Ala Pro Asn Leu Glu Gly
225 230 235 240

Gly Pro Ser Val Phe Ile Phe Pro Pro Asn Ile Lys Asp Val Leu Met
 245 250 255
 5
 Ile Ser Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Val Ser Glu
 260 265 270
 10
 Asp Asp Pro Asp Val Arg Ile Ser Trp Phe Val Asn Asn Val Glu Val
 275 280 285
 15 His Thr Ala Gln Thr Gln Thr His Arg Glu Asp Tyr Asn Ser Thr Ile
 290 295 300
 Arg Val Val Ser Ala Leu Pro Ile Gln His Gln Asp Trp Met Ser Gly
 20 305 310 315 320
 Lys Glu Phe Lys Cys Lys Val Asn Asn Lys Asp Leu Pro Ser Pro Ile
 325 330 335
 25
 Glu Arg Thr Ile Ser Lys Ile Lys Gly Leu Val Arg Ala Pro Gln Val
 340 345 350
 30
 Tyr Ile Leu Pro Pro Pro Ala Glu Gln Leu Ser Arg Lys Asp Val Ser
 355 360 365
 35 Leu Thr Cys Leu Val Val Gly Phe Asn Pro Gly Asp Ile Ser Val Glu
 370 375 380

Trp Thr Ser Asn Gly His Thr Glu Glu Asn Tyr Lys Asp Thr Ala Pro
 385 390 395 400
 5

Val Leu Asp Ser Asp Gly Ser Tyr Phe Ile Tyr Ser Lys Leu Asp Ile
 405 410 415

10
 Lys Thr Ser Lys Trp Glu Lys Thr Asp Ser Phe Ser Cys Asn Val Arg
 420 425 430

15 His Glu Gly Leu Lys Asn Tyr Tyr Leu Lys Lys Thr Ile Ser Arg Ser
 435 440 445

20 Pro Gly Lys
 450

25 <210> 86
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 <212> PRT
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30 <220>
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 <400> 86

35 Gln Ala Ser Gln Gly Thr Ser Ile Asn Leu Asn
 1 5 10

<210> 87
<211> 7
<212> PRT
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<220>
<223> C10.2 CDR 2 Light Chain

<400> 87

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Gly Ala Ser Asn Leu Glu Asp
1 5

15

<210> 88
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<212> PRT
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<220>
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<400> 88

25

Leu Gln His Thr Tyr Leu Pro
1 5

<210> 89

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<211> 13
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<220>
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<223> C10.2 CDR 1 Heavy Chain

<400> 89

 Lys Ala Ser Gly Tyr Thr Phe Thr Asp Arg Thr Ile His
 1 5 10
 5

 <210> 90
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 10 <213> Artificial

 <220>
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 15 <400> 90

 Tyr Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Asn Phe Lys
 1 5 10 15

 20
 Gly

 25 <210> 91
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 <212> PRT
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 30 <220>
 <223> C10.2 CDR 3 Heavy Chain

 <400> 91

 35 Arg Gly Ala Met Asp Tyr
 1 5

<210> 92
 <211> 214
 5 <212> PRT
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 <220>
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 10
 <400> 92

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

Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
 100 105 110

5 Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
 115 120 125

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile
 10 130 135 140

Asn Val Lys Trp Lys Ile Asp Gly Ser Glu Arg Gln Asn Gly Val Leu
 145 150 155 160

15 Asn Ser Trp Thr Asp Gln Asp Ser Lys Asp Ser Thr Tyr Ser Met Ser
 165 170 175

20 Ser Thr Leu Thr Leu Thr Lys Asp Glu Tyr Glu Arg His Asn Ser Tyr
 180 185 190

25 Thr Cys Glu Ala Thr His Lys Thr Ser Thr Ser Pro Ile Val Lys Ser
 195 200 205

30 Phe Asn Arg Asn Glu Cys
 210

<210> 93
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 35 <212> PRT
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5 <400> 93

Gln Val Gln Leu Gln Gln Ser Asp Ala Glu Leu Val Lys Pro Gly Ala
 1 5 10 15

10

Ser Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Arg
 20 25 30

15

Thr Ile His Trp Val Lys Gln Arg Pro Glu Gln Gly Leu Glu Trp Ile
 35 40 45

20

Gly Tyr Ile Tyr Pro Gly Asp Gly Ser Thr Lys Tyr Asn Glu Asn Phe
 50 55 60

25

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
 65 70 75 80

Met Gln Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
 85 90 95

30

Ala Arg Arg Gly Ala Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr
 100 105 110

35

Val Ser Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro
 115 120 125

5 Gly Ser Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val
130 135 140

10 Lys Gly Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser
145 150 155 160

15 Leu Ser Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu
165 170 175

20 Tyr Thr Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser
180 185 190

25 Glu Thr Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val
195 200 205

30 Asp Lys Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys
210 215 220

35 Thr Val Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys
225 230 235 240

40 Asp Val Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val
245 250 255

45 Asp Ile Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp
260 265 270

Asp Val Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe
275 280 285

5

Asn Ser Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp
290 295 300

10

Trp Leu Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe
305 310 315 320

15

Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys
325 330 335

20

Ala Pro Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys
340 345 350

25

Asp Lys Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp
355 360 365

30

Ile Thr Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys
370 375 380

35

Asn Thr Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser
385 390 395 400

35

Lys Leu Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr
405 410 415

Cys Ser Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser
420 425 430

5

Leu Ser His Ser Pro Gly Lys
435

10

<210> 94
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<212> PRT
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15

<220>
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<400> 94

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Ser Ala Ser Ser Ser Val Ser Phe Met His
1 5 10

25

<210> 95
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<212> PRT
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30

<220>
<223> CDR1 VL 7A10/8D9

<400> 95

35

Ser Ala Ser Ser Ser Val Ser Tyr Ile His
1 5 10

<210> 96
<211> 9
5 <212> PRT
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<400> 96

Gln Gln Trp Thr Ser Asn Pro Pro Phe
1 5
15

<210> 97
<211> 5
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<220>
<223> CDR1 VH 7C4

25 <400> 97

Arg Tyr Trp Met His
1 5

30
<210> 98
<211> 17
<212> PRT
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35
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<223> CDR2 VH 5A1

<400> 98

5 Arg Val Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe Lys
1 5 10 15

Thr

10

<210> 99
<211> 17
15 <212> PRT
<213> artificial

<220>
<223> CDR2 VH 9G11

20

<400> 99

Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val His Phe Lys
1 5 10 15

25

Thr

30

<210> 100
<211> 17
<212> PRT
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35

<220>

<223> CDR2 VH 9C12

<400> 100

5 Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Ile Lys
1 5 10 15

Thr

10

<210> 101
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15 <212> PRT
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<220>
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20

<400> 101

Leu Gly His Tyr Gly Asn Leu Asn Ala Met Asp Tyr
1 5 10

25

<210> 102
<211> 12
<212> PRT
30 <213> Artificial

<220>
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35 <400> 102

Leu Gly His Tyr Ser Lys Val Leu Ala Met Asp Tyr
 1 5 10

5 <210> 103
 <211> 12
 <212> PRT
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10 <220>
 <223> CDR3 VH 7A10/8D9
 <400> 103

15 Leu Gly His Tyr Gly Asn Leu Tyr Ala Lys Asp Tyr
 1 5 10

20 <210> 104
 <211> 106
 <212> PRT
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 25 <223> 5A1 VL
 <400> 104

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 30 1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30
 35

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45

5 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Arg Leu Glu Pro Glu
 10 65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

15

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

20

<210> 105
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 <212> PRT
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25

<220>
 <223> 5A1 VH

<400> 105

30

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Gln Gly Ala
 1 5 10 15

35 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

Trp Met His Tyr Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 5

Gly Arg Val Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

10

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Thr Thr Ser Thr Ala Tyr
 65 70 75 80

15 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Leu Gly His Tyr Gly Asn Leu Asn Ala Met Asp Tyr Trp Gly
 20 100 105 110

Gln Gly Thr Leu Val Thr
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25

<210> 106
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 <212> PRT
 30 <213> artificial

<220>
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35 <400> 106

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

5 Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 10 35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

15

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

20

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

25 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

30 <210> 107
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<220>
 35 <223> 9D7 VH

<400> 107

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 5

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

10
 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

15 Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 20 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

25

Ala Arg Leu Gly His Tyr Ser Lys Val Leu Ala Met Asp Tyr Trp Gly
 100 105 110

30

Gln Gly Thr Leu Val Thr
 115

35 <210> 108

<211> 106

<212> PRT

<213> artificial

<220>

5 <223> 9G11 VL

<400> 108

10 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15

 Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
 20 25 30
 15

 His Trp Tyr Gln Gln Lys Gln Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45
 20

 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

25 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95
 30

 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105
 35

<210> 109

<211> 118

<212> PRT

<213> artificial

5

<220>

<223> 9G11 VH

<400> 109

10

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

15 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

20 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

25 Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val His Phe
 50 55 60

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

30 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

35 Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Thr Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr
 115
 5
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 <212> PRT
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 15 <400> 110
 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 20
 Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Phe Met
 20 25 30
 25 His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45
 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 30 50 55 60
 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80
 35

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

5 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

<210> 111
 10 <211> 118
 <212> PRT
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 15 <223> 7C4 VH

<400> 111

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 20 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Arg Tyr
 20 25 30

25

Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Pro Glu Trp Ile
 35 40 45

30

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

35 Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

5

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
100 105 110

10

Gln Gly Thr Leu Val Thr
115

15 <210> 112
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20 <220>
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25 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
30 20 25 30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
35 40 45

35

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 50 55 60

5 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 10 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

15

<210> 113
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 <212> PRT
 20 <213> artificial

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25 <400> 113

Gln Val Gln Leu Val Gln Gln Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

30

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

35 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60
 5

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

10

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

15 Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
 100 105 110

Gln Gly Thr Leu Val Thr
 20 115

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 25 <212> PRT
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 30

<400> 114

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 35

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Ile
 20 25 30

5 His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
 35 40 45

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
 10 50 55 60

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

15 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Thr Ser Asn Pro Pro Asn
 85 90 95

20 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

25 <210> 115
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30 <220>
 <223> 7A10 VH
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35 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30
 5

Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45
 10

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60
 15

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80
 20

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 25

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Lys Asp Tyr Trp Gly
 100 105 110
 30

Gln Gly Thr Leu Val Thr
 115

35

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 <212> PRT
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 35

<220>

<223> 8D9 VL

<400> 116

5 Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

10 Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Ile
20 25 30

15 His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Trp Ile Tyr
35 40 45

20 Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

25 Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
65 70 75 80

30 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
85 90 95

35 Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 117

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35 <212> PRT

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<220>

<223> 8D9 VH

5 <400> 117

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

10

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

15

Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

20

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

25

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

30

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Lys Asp Tyr Trp Gly
 100 105 110

35

Gln Gly Thr Leu Val Thr
 115

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 5 <212> PRT
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 10
 <400> 118

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

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105

5 <210> 119
 <211> 118
 <212> PRT
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10 <220>
 <223> 9C12 VH
 <400> 119

15 Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15

20 Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

25 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Ile
 50 55 60

30 Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
 65 70 75 80

35 Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95

Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
100 105 110

5

Gln Gly Thr Leu Val Thr
115

10

<210> 120
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<212> PRT
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15

<220>
<223> 6B6 VL

<400> 120

20

Glu Ile Val Leu Thr Gln Ser Pro Ala Thr Leu Ser Leu Ser Pro Gly
1 5 10 15

25

Glu Arg Ala Thr Leu Ser Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

30

His Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Arg Leu Ile Tyr
35 40 45

35

Asp Thr Ser Lys Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Asp Tyr Thr Leu Thr Ile Ser Ser Leu Glu Pro Glu
 65 70 75 80

5 Asp Phe Ala Val Tyr Tyr Cys Gln Gln Trp Ser Ser Asn Pro Pro Thr
 85 90 95

Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 10 100 105

<210> 121
 <211> 118
 15 <212> PRT
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<220>
 <223> 6B6 VH
 20 <400> 121

Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys Pro Gly Ala
 1 5 10 15
 25

Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
 20 25 30

30 Trp Met His Trp Met Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Ile
 35 40 45

35 Gly Arg Ile Asp Pro Asn Ser Gly Thr Thr Lys Tyr Asn Val Asn Phe
 50 55 60

Lys Thr Arg Ala Thr Leu Thr Val Asp Lys Ser Thr Ser Thr Ala Tyr
65 70 75 80
5

Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys
85 90 95

10
Ala Arg Leu Gly His Tyr Gly Asn Leu Tyr Ala Met Asp Tyr Trp Gly
100 105 110

15 Gln Gly Thr Leu Val Thr
115

20

CLAIMS

1. Use of an alpha-synuclein binding monoclonal antibody, or an antigen-binding fragment thereof in the manufacture of a medicament for inhibiting aggregation of tau wherein said alpha-synuclein antibody is to be administered to a patient with Alzheimer's disease and wherein the patient with Alzheimer's disease does not have Lewy body variant of Alzheimer's disease or combined Parkinson and Alzheimer's disease and wherein the alpha-synuclein antibody, or an antigen-binding fragment thereof, is selected from the group consisting of antibodies (1)-(5):.
- Antibody (1)** or an antigen-binding fragment thereof, comprising:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
 - (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:2**;
 - (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
 - (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
 - (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and
 - (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;
- Antibody (2)** or an antigen-binding fragment thereof, comprising:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
 - (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:33**;
 - (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
 - (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
 - (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and

(f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

Antibody (3) or an antigen-binding fragment thereof, comprising:

5 (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;

(b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:34**;

10 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;

(d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;

(e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and

15 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

Antibody (4) or an antigen-binding fragment thereof, comprising:

20 (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;

(b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:35**;

(c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;

25 (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;

(e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**; and

30 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

and

Antibody (5) or an antigen-binding fragment thereof, comprising:

- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:20**;
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:21**;
- 5 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:22**;
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:23**;
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:24**; and
- 10 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:25**.