

Agents, Uses and Methods for the Treatment of Synucleinopathy

Field of the Invention:

- 5 **[0001]** The present invention relates to a class of monoclonal antibody that specifically binds to alpha-synuclein for use in combinational therapies, as well as to methods of using these in the treatment of synucleinopathies.

Reference To Sequence Listing:

- [0002]** This application includes one or more Sequence Listings pursuant to 37
10 C.F.R. 1.821 et seq., which are disclosed in computer-readable media (file name: 1069_ST25.txt, created on 15 November 2016, and having a size of 41 kB), which file is herein incorporated by reference in its entirety.

Background of the Invention:

- [0003]** Synucleinopathies, also known as Lewy body diseases (LBDs), are
15 characterized by deposition of intracellular protein aggregates that are microscopically visible as Lewy bodies (LBs) and/or Lewy neurites, where the protein alpha-synuclein is the major component (Jellinger, Mov Disord. 2012 Jan;27(1):8-30; McKeith et al., Neurology (1996) 47:1113-24). Synucleinopathies include Parkinson's disease (PD) (including idiopathic and inherited forms of Parkinson's disease) and Diffuse Lewy
20 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 (CAPD), pure autonomic failure (PAF) and multiple system atrophy (MSA; e.g., Olivopontocerebellar Atrophy, Striatonigral Degeneration and Shy-Drager Syndrome)). Synucleinopathies frequently have degeneration of the dopaminergic nigrostriatal
25 system, responsible for the core motor deficits in Parkinsonism (rigidity, bradykinesia, resting tremor), but there is also widespread occurrence of Lewy bodies and dystrophic Lewy neurites in the central, peripheral and autonomic nervous system and brain regions and other organs associated with non-motor dysfunctions, such as dementia and autonomic nervous system deficits. Several of the non-motor signs and symptoms
30 are thought to precede motor symptoms in Parkinson's disease and other

synucleinopathies. Such early signs include, for example, REM sleep behaviour disorder (RBD) and loss of smell and constipation (Mahowald et al., *Neurology* (2010) 75:488-489). Synucleinopathies continue to be a common cause for movement disorders and cognitive deterioration in the aging population (Galasko et al., *Arch. Neurol.* (1994) 51:888-95).

[0004] 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 affecting neural communication, plasticity, learning and memory.

[0005] Several studies have implicated alpha-synuclein with a central role in PD pathogenesis. The protein can aggregate to form intracellular insoluble fibrils in pathological conditions. For example, synuclein accumulates in LBs (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). An important finding has been that alpha-synuclein can be secreted into the extracellular fluid and be present in plasma and cerebrospinal fluid (CSF). Several studies, for example by Pacheco et al. (2015) and others (Pacheco et al. *J Neurochem.* 2015 Mar;132(6):731-4; Conway et al., *Proc Natl Acad Sci USA* (2000) 97:571-576; Volles et al., *J. Biochem.* 42:7871-7878, 2003) have suggested that extracellular-synuclein plays a pathogenic role in the brain. They demonstrated that extracellular alpha-synuclein oligomers possesses neurotoxicity toward brain neuronal plasma membranes. 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. 2014, *Nat Rev Neurol.* 2014 Feb;10(2):92-8; Hansen and Li 2012, *Trends Mol Med.* 2012 May;18(5):248-55). These findings have given rise to a hope that extracellular-synuclein could be targeted by immunotherapy (Vekrellis et al. 2011, *Lancet Neurol.* 2011 Nov;10(11):1015-25).

[0006] Naturally occurring alpha-synuclein auto-antibodies have been shown to be present in both PD patients and healthy controls (Smith et al. 2012, *PLoS One.* 2012;7(12):e52285; Maetzler et al. 2014, *PLoS One.* 2014 Feb 21;9(2):e88604,

Papachroni et al. 2007 J Neurochem. 2007 May;101(3):749-56 and Woulfe et al. 2002, Neurology. 2002 May 14;58(9):1435-6), sometimes increased levels of auto-antibodies to alpha-synuclein in PD (Gruden et al. 2011, J Neuroimmunol. 2011 Apr;233(1-2):221-7, Gruden et al. 2012, Neuroimmunomodulation. 2012;19(6):334-42 and Yanamandra
 5 2011, PLoS One. 2011 Apr 25;6(4):e18513) or decreased auto-antibodies to alpha-synuclein in PD patients compared to healthy controls have been reported (Besong-Agbo et al 2013, Neurology. 2013 Jan 8;80(2):169-75). . The possibility that circulating anti-alpha-synuclein autoantibodies may serve a protective role with respect to alpha-synuclein aggregation was suggested very early on after finding of the auto-antibodies
 10 (Woulfe et al. 2002, Neurology. 2002 May 14;58(9):1435-6).

[0007] Over expression of alpha-synuclein in transgenic mice mimics some pathological aspects of Lewy body disease. Several different transgenic lines of mice over-expressing alpha-synuclein have been generated in the last ten years (described in reviews: Koehler et al 2014, PLoS One. 2013 May 31;8(5):e64649; Fleming and
 15 Chesselet, 2006, Behav Pharmacol. 2006 Sep;17(5-6):383-91; Springer and Kahle 2006, Curr Neurol Neurosci Rep. 2006 Sep;6(5):432-6). Mouse lines with Thy-1 and PDGF-beta promoters develop motor deficits and cognitive deficits and have been used to demonstrate a neuroprotective effect of antibodies directed against alpha-synuclein in vivo. However, none of the transgenic lines have robust degeneration of
 20 dopaminergic neurons, and often the motor phenotypes are driven by expression in motor neurons, which do not normally degenerate in Parkinson's disease. Therefore, it is not clear if positive outcome of a potential disease modifying treatment is mediated through effects on dopaminergic neurons or other central nervous system neurons.

[0008] One robust finding in the transgenic mouse models has been that chronic
 25 overexpression of human alpha-synuclein impairs synaptic function. Using studies in both in vitro and in vivo systems it was shown that overexpression of wild-type (wt) human alpha-synuclein impaired synaptic transmission in hippocampus (Nemani et al. 2010, Neuron. 2010 Jan 14;65(1):66-79; Paumier et al. 2013, PLoS One. 2013 Aug 1;8(8):e70274). This was shown in the CA1 region of the hippocampus where both
 30 studies found reduced basal synaptic transmission. The mechanism behind this was assumed to be intracellular accumulation of alpha-synuclein leading to dysfunctional synaptic release. However, the recent findings about secretion of alpha-synuclein into extracellular space in synapses and the toxic effects of alpha-synuclein oligomers on

synapse function opens for the possibility of a role of extracellular alpha-synuclein in synaptic dysfunction, and as such for the ability of therapeutic antibodies to rescue the deficit.

[0009] The use of viral vectors to over-express alpha-synuclein represents an important way to model PD in rodents because this approach produces a relative fast progressive degeneration of nigrostriatal neurons, a feature not yet reproduced by genetic mutations in mice or rats (Kirik and Bjorklund, 2003, Trends Neurosci. 2003 Jul;26(7):386-92). Furthermore, viral gene delivery revealed the ability of wt alpha-synuclein to induce nigrostriatal pathology (Kirik et al. 2002, J Neurosci. 2002 Apr 1;22(7):2780-91), a finding in agreement with evidence in familial forms of PD with alpha-synuclein duplications and triplications (Lee and Trojanowski, 2006, Neuron. 2006 Oct 5;52(1):33-8). In one study, it has been shown that a pool of goat antibodies against the alpha-synuclein N-terminal protected against dopaminergic cell death and ameliorated behavioural deficits in a AAV-alpha-synuclein based rat model of Parkinson's disease (Shahaduzzaman et al 2015, PLoS One. 2015 Feb 6;10(2):e0116841).

[00010] Prion like spreading of alpha-synuclein pathology has recently been shown to develop alpha-synuclein pathology and also develop dopaminergic cell death (Luk et al. 2012, Science. 2012 Nov 16;338(6109):949-53). This model has been used to show that alpha-synuclein antibodies are able to ameliorate the pathology (Tran *et al.* 2014, Cell Rep. 2014 Jun 26;7(6):2054-65). In this model antibody treatment was able to reduce accumulation of phosphorylated alpha-synuclein in several brain regions – including dopaminergic neurons in substantia nigra, and reduce development of motor deficit.

[00011] 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 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.

[00012] Overall the accumulation of alpha-synuclein with similar morphological and neurological alterations in animal models as diverse as humans, mice, and flies
5 suggests that this molecule is central in the pathogenesis of Lewy body diseases.

[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
10 motor and cognitive deficits in transgenic mice (Games et al. 2014, J Neurosci. 2014 Jul 9;34(28):9441-54). 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. A C-terminal antibody 274 which targets an epitope that involves alpha-synuclein residues
15 120-140 (Bae et al. 2012, J Neurosci. 2012 Sep 26;32(39):13454-69) was also shown to have an effect in a 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 (Lindström et al. 2014, Neurobiol Dis.
20 2014 Sep;69:134-43 and Spencer et al. 2014, Mol Ther. 2014 Oct;22(10):1753-67). 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
25 Dis. 2015;79:81-99).

[00014] As the toxic form of alpha-synuclein is unknown, a therapeutic antibody should be ideally able to bind to most of the alpha-synuclein species that are formed by alternative splicing or posttranslational modifications, such as truncations, as well as oligomeric and fibrillary forms. One problem with current antibodies that have been
30 tested as therapeutics in preclinical models, as discussed above, is that many of them target C-terminal epitopes, which are not found in some of the major truncated forms of alpha-synuclein. For example, the amino acids that are important for binding of 9E4 are

asparagine 122 and tyrosine 125 (according to an alanine scan presented in patent US20140127131), and this means that this antibody cannot bind alpha-synuclein which is truncated at amino acids 119, and 122, which are some of the major truncated species in Parkinson brain tissue (Kellie *et al.* Sci Rep. 2014;4:5797). The same would
5 be the case for the antibody 274 and antibody mab47 (US8,632,776). Also, amino terminal antibodies would possibly not be able to bind to some of the major truncated species that lack the first amino acids of alpha-synuclein, such as alpha-synuclein truncated to amino acids 5-140. For the 9E4 antibody, one suggested mechanism of action is the prevention of truncation at amino acids 119-122 in extracellular space, as
10 the antibody will bind to the same region where the protease that will cleave alpha-synuclein (Games *et al.* 2014, J Neurosci. 2014 Jul 9;34(28):9441-54). A similar mechanism of action could also be found with antibodies in close proximity of the site, and therefore many antibodies around this region would be expected to have this activity.

15 **[00015]** There is some support for a toxic role of the truncated alpha-synuclein species in animal models. Expression of truncated alpha-synuclein under the tyrosine-hydroxylase promoter has been shown to lead to nigrostriatal pathology, which is normally not seen in transgenic alpha-synuclein models (Tofaris *et al.* 2006, J Neurosci. 2006 Apr 12;26(15):3942-50; Wakamatsu *et al.* 2006, Neurobiol Aging. 2008
20 Apr;29(4):574-85). For example, expression of amino acids 1–130 of a human alpha-synuclein protein having the A53T mutation caused embryonic loss of dopaminergic neurons in the substantia nigra pars compacta whereas expression of the full length protein did not (Wakamatsu *et al.* 2006, Neurobiol Aging. 2008 Apr;29(4):574-85). Expression of a 120 amino acid alpha-synuclein molecule under the
25 calcium/calmodulin-dependent protein kinase II alpha (CamKII-alpha) promoter was associated with alpha-synuclein aggregation and a progressive deficit in cortical-hippocampal memory tests including the Barnes maze and novel object recognition (Hall *et al.* 2015, Exp Neurol. 2015 Feb;264:8-13). Also in the rat AAV model co-expression of C-terminal truncated alpha-synuclein enhanced full-length alpha-
30 synuclein-induced pathology (Ulusoy *et al.* 2010, Eur J Neurosci. 2010 Aug;32(3):409-22).

[00016] In this invention, antibodies (such as “GM37” and “GM285”, described in the Examples) have been generated that can bind to the toxic alpha-synuclein fragment 1-

119/122 and neutralize this truncated form of alpha-synuclein. The antibodies of the invention, such as GM37 and GM285, are capable of binding to other oligomeric forms of alpha-synuclein and altering their uptake by other CNS resident cells in a manner that reduce the spreading of disease. Furthermore, the antibodies of the invention,
5 such as GM37 and 285, were surprisingly found to be superior to prior art antibodies such as 9E4 in binding to different alpha-synuclein species in human brain, and has a surprising superior effect on clearing extracellular alpha-synuclein and normalising impaired synaptic transmission induced by the presence of abnormal alpha-synuclein in vivo. Further illustrating their therapeutic capabilities, the antibodies of the invention,
10 such as GM37 and 285, are able to prevent the appearance of a disease related motor phenotype in a rat model for Parkinson's disease. Finally, antibodies GM37 and GM285 are able to inhibit seeding of aggregation and phosphorylation of endogenous alpha-synuclein induced by extracellular added recombinant pathological alpha-synuclein seeds in primary mouse neurons. Antibodies such as GM37 and 285 can
15 also inhibit seeding of alpha-synuclein pathology into dopaminergic neurons in vivo using a mouse model for Parkinson's disease, further supporting the therapeutic capability of these antibodies in preventing the cell to cell propagation of pathology. Together these data strongly support the use of these antibodies, GM37 and GM285, as new therapeutic agents capable of modifying disease through inhibition of the
20 mechanism by which the disease pathology spreads between the neurons Parkinson's patients.

[00017] In a further aspect of the invention is provided 3 amino acid variants of the GM37 antibody. All the variants have similar functional readouts as the parent antibody, GM37, but with improved properties for manufacturability. The variants
25 reduce the risk of post-translational modification occurring within the binding domain of the GM37 antibody and provide some improvement in the production of the antibody. This is advantageous because large scale clinical or commercial manufacturing of antibodies is complicated and expensive, and providing a homogenous product in pharmaceutical medicaments is crucial in particular for immunoglobulins and proteins.

30 **[00018]** The antibodies of the invention, GM285, GM37 and its variants, may be used in combination with further medicaments as disclosed herein to improve the efficacy, safety and compliance of treatment

- [00019]** The combinational treatments may be with symptomatic drugs such as dopamine (Pramipexole) (Loiodice et al. Psychopharmacology (Berl). 2016 Sep 10.; Luo HT et al F. Exp Ther Med. 2016 Sep;12(3):1373-1376) or a combination with a disease modifying compound such as caspase inhibitors (e.g. prodrug VX-765; Pralnacasan) (Bassil F et al Proc Natl Acad Sci U S A. 2016 Aug 23;113(34):9593-8; Wang et al Proc Natl Acad Sci U S A. 2016 Aug 23;113(34):9587-92) or a Calpain inhibitor (e.g. Calpeptin (ABT-957 from AbbVie) (Czapski et al FEBS Lett. 2013 Sep 17;587(18):3135-41), a LRRK2 inhibitor (e.g. PF-06447475 (Daher et al J Biol Chem. 2015 Aug 7;290(32):19433-44), a BACE1 inhibitor (e.g. NB-360) (Spencer et al J Neurosci. 2016 Jul 27;36(30):7971-84; Schelle et al Alzheimers Dement. 2016 Oct 14. Pii), an aggregation inhibitor (e.g. neuropore NPT100-18A (NPT200-11) (Wrasidlo et al E. Brain. 2016 Sep 27. pii: aww238); EGCG (Xu et al Neurochem Res. 2016 Oct;41(10):2788-2796); an antibody recognising any form of A β (e.g. Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab (Guerrero-Muñoz et al Neurobiol Dis. 2014 Nov;71:14-23; Kabiraj et al ACS Chem Neurosci. 2016 Oct 3; Hepp et al J Neuropathol Exp Neurol. 2016 Oct;75(10):936-945; Walker et al Acta Neuropathol. 2015 May;129(5):729-48.; an Inflammation inhibitor (e.g. C16H15NO4 (C29) (Kim et al Exp Neurobiol. 2016 Jun;25(3):113-9; Kim et al J Neuroinflammation. 2015 Dec 18;12:236.; He et al Eur J Neurosci. 2016 Jan;43(1):41-52; Regloid et al Prog Neurobiol. 2015 Nov 2. pii: S0301-0082(15)00128-8.); a LAG-3 antibody (e.g. BMS-986016, GSK2831781 or IMP321) (Mao et al Science. 2016 Sep 30;353(6307); small molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia - drug-like phenyl-sulfonamide compound (ELN484228), that targets α -synuclein (Tóth et al PLoS One. 2014 Feb 14;9(2):e87133. Erratum in: PLoS One. 2014;9(5); Gardai et al, PLoS One. 2013 Aug 23;8(8); Isradipine (Surmeier et al Biochem Biophys Res Commun. 2016 Aug 30. pii: S0006-291X(16)31432-2.; Emanuele et al EBioMedicine. 2016 May;7:191-204; Di Scala et al Sci Rep. 2016 Jun 29); Nicotine or Caffeine (Tavassoly et al Eur J Med Chem. 2014 Dec 17;88:42-54; Derkinderen et al Mov Disord. 2014 Jul;29(8):976-9); a modulator of GCase (e.g. NCGC607, a small-molecule noninhibitory chaperone of glucocerebrosidase; Miglustat, an inhibitor of GlcCer synthase and Eliglustat (Cerdelga) a substrate reduction therapy) (Mazzulli et al J Neurosci. 2016 Jul 20;36(29):7693-706; Yap et al J Biol Chem. 2011 Aug 12;286(32):28080-8; Yap et al Mol Genet Metab. 2013 Jan;108(1):56-64; or commercial available drugs like MAO-B inhibitors,

Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A2A antagonists such as for example Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.

Summary of the Invention:

5 **[00020]** The invention relates to combinational treatments using monoclonal antibodies, and antigen-binding fragments thereof, capable of specifically binding an epitope within amino acids 112-117 in alpha-synuclein (**SEQ ID NO:9** (ILEDMP)) and an additional medicament. The epitope bound by the antibodies or antibody-binding fragments thereof of the invention, such as exemplary antibody "GM37", or "GM285", is
10 referred to herein as "the 112-117 epitope". The antibodies of the present invention specifically bind to an epitope within the 112-117 epitope and may, according to one embodiment, compete with antibody GM37 or GM285 for binding to an epitope within amino acids 112-117. For example, antibodies or antigen-binding fragments thereof according to the invention may compete for binding to an epitope within amino acids
15 112-117 of human alpha-synuclein with 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**. Such competitive binding inhibition can be determined using assays and methods well known in the art, for example using an unlabelled binding assay such as surface plasmon resonance (SPR). For example, immobilising human alpha-synuclein on a
20 surface and incubating with or without the reference antibody 'GM37' prior to incubation with an antibody or binding fragment to be tested. Alternatively, a pair-wise mapping approach can be used, in which the reference antibody 'GM37' is immobilised to the surface, human alpha-synuclein antigen is bound to the immobilised antibody, and then a second antibody is tested for simultaneous binding ability to human alpha-synuclein
25 (see 'BIAcore® Assay Handbook', GE Healthcare Life Sciences, 29-0194-00 AA 05/2012; the disclosures of which are incorporated herein by reference).

[00021] More specifically the GM285 antibody binds an epitope within residues 112-117 of alpha-synuclein comprising residues 112-115 of alpha-synuclein (ILED; **SEQ ID NO:19**).

30 **[00022]** In one embodiment, the invention relates to combinational treatments with monoclonal antibody GM37, its variants (e.g., GM37 Variant 1, GM37 Variant 2 and GM37 Variant 3), or GM285.

[00023] In particular, the invention provides combinational treatments with a monoclonal antibody GM37, its variants (e.g., GM37 Variant 1, GM37 Variant 2 and GM37 Variant 3), or GM285, and encompasses such antibodies as well as derivatives thereof that possess a sufficient number (e.g., 1, 2, or 3) light chain CDRs and a
 5 sufficient number (e.g., 1, 2, or 3) heavy chain CDRs to form a binding site capable of specifically binding to human synuclein. Preferably, such antibodies will possess the three light chain CDRs and three heavy chain CDRs, as defined below. The numbering of amino acid residues in this region is according to IMGT®, the international ImMunoGeneTics information system® or, Kabat, E. A., Wu, T. T., Perry, H. M.,
 10 Gottesmann, K. S. & Foeller, C. (1991). Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services; Chothia, C. & Lesk, A. M. (1987). Canonical structures For The Hypervariable domains Of Immunoglobulins. J. Mol. Biol. 196, 901-917.

[00024] In one embodiment, the monoclonal antibody or antigen-binding fragments
 15 thereof possesses a synuclein antigen-binding fragment comprising or consisting of:

- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:1**;
and/or
- (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:2**;
and/or
- 20 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
and/or
- (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:4**;
and/or
- (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:5**;
25 and/or
- (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

that is capable of specifically binding to human alpha-synuclein.

[00025] In another embodiment, the monoclonal antibody or antigen-binding fragments thereof possesses a synuclein antigen-binding fragment comprising or consisting of:

- 30 (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**,
34 or 35;
- (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**;
 that is capable of specifically binding to human alpha- synuclein.
- 5 **[00026]** In yet another embodiment, the monoclonal antibody or antigen-binding fragments thereof possesses a synuclein antigen-binding fragment comprising or consisting of:
- (a) a Heavy Chain CDR1 having the amino acid sequence of **SEQ ID NO:20**;
 and/or
 10 (b) a Heavy Chain CDR2 having the amino acid sequence of **SEQ ID NO:21**;
 and/or
 (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:22**;
 and/or
 (d) a Light Chain CDR1 having the amino acid sequence of **SEQ ID NO:23**;
 15 and/or
 (e) a Light Chain CDR2 having the amino acid sequence of **SEQ ID NO:24**;
 and/or
 (f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:25**.
 that is capable of specifically binding to human alpha-synuclein.
- 20 **[00027]** The antibodies and antigen-binding fragments thereof of the invention may be used in combinational treatments in a method to treat synucleinopathies, such as Parkinson's disease ((PD), including idiopathic and inherited forms of Parkinson's disease), Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Gauchers Disease (GD), Combined Alzheimer's and Parkinson disease
 25 (CAPD), pure autonomic failure and multiple system atrophy in combination with an additional medicament selected from the group comprising dopamine, caspase inhibitors, calpain inhibitors, LRRK2 inhibitors, BACE1 inhibitors, aggregation inhibitors, antibodies against any form of Amyloid-beta, inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity
 30 of microglia, nicotine, caffeine, modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A2A antagonists.

- [00028]** In one embodiment the medicament may be selected from the group comprising pramipexole, VX-765 (Pralnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzumab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, C16H15NO₄, BMS-986016,
- 5 GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.

Brief Description of the Drawings

- [00029]** Fig. 1 shows immunization protocols for generation of hybridomas. The table
- 10 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-
- 15 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**).
- 20 **[00030]** Fig. 2 (**PANEL A-C**) shows screening of GM37 for binding to alpha synuclein, alpha-synuclein homologs and orthologs.
- A) Binding of antibody GM37 to alpha-synuclein using a no wash solution based ELISA (FMAT).
- B) Using SPR (Fortebio) binding of antibody GM37 is specific for alpha-synuclein
- 25 (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**).
- 30 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**).

[00031] Fig. 3 (Panels A-C) shows real time binding Affinity of GM37

- 5 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.
- 10 B) Signal from binding at different concentrations converted into a binding curve.
- C) Calculated binding constants of antibody GM37 (denoted hlgG1-6004-037-C106S) (**Example 2**).

[00032] Fig. 4 (Panels A-C) shows real time binding Affinity of GM285

- 15 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.
- 20 B) Signal from binding at different concentrations converted into a binding curve.
- C) Calculated binding constants of antibody GM285 (denoted hlgG1-6004-285) (**Example 2**).

[00033] Fig. 5 (Panels A-C) shows real time binding of comparator antibody 9E4

- 25 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.
- 30 B) Signal from binding at different concentrations converted into a binding curve.
- C) Calculated binding constants for antibody 9E4. (**Example 2**).

[00034] Fig. 6 shows the amino acid sequence of alpha-synuclein. Major truncation sites (indicated by arrows) identified by mass spectrometry in human brain tissue (Kellie JF, Higgs RE, Ryder JW, Major A, Beach TG, Adler CH, Merchant K, Knierman MD. Quantitative measurement of intact alpha-synuclein proteoforms from post-mortem control and Parkinson's disease brain tissue by mass spectrometry. Sci Rep. 2014 Jul 23;4:5797. doi: 10.1038/srep05797)

[00035] Fig. 7 (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).
 A) GM37 epitope requires peptide sequence ILEDMP (**SEQ ID NO:9**) for full binding.
 B) GM285 requires peptide ILED (**SEQ ID NO:19**) for full binding. (**Example 3**).

[00036] Fig. 8 (Panels A-B) shows a schematic representation of truncated forms of alpha-synuclein.
 A) binding epitopes of GM37/285 (ILEDMP; **SEQ ID NO:9**) and 9E4 (NEAYE; **SEQ ID NO:36**) are shown in bold on the alpha-synuclein amino acid sequence (**SEQ ID NO:10**). Arrows indicates the c-terminal truncations sites from Fig 6.
 B) Major truncated forms of alpha-synuclein that have been identified from human brain material. Size based on amino acid numbers is indicated on the right side. Full length alpha-synuclein is 140 amino acids. As can be deduced from the epitopes, GM37, it's variants 1-3, and GM285 should bind full length and the 1-119/122, 1-135 fragments. Antibody 9E4 will bind only to full length and 1-135 fragment. The specific nature of the smaller c-terminal fragments left after the truncations are not shown.

[00037] Fig. 9 shows that antibodies GM37 and GM285 immunoprecipitate full length alpha-synuclein as well as truncated alpha-synuclein from human brain. Crude homogenates of human DLB brain were incubated with the test antibodies (Beads (No ab), B12-human IgG1 control antibody not binding to alpha-synuclein, GM-37, GM37 variant 2, GM-285 and murine (m)9E4) and the immunodepleted supernatant and immunoprecipitated material was separated on SDS-PAGE. The western blot shows the bands representing the full length and the different truncated forms of alpha-synuclein being depleted from the supernatant and being immunoprecipitated with the antibodies (IP). As can be seen, the GM37, GM37v2 and GM285 antibody depleted the

major alpha-synuclein species from the supernatant, and the IP shows these species, the truncated species 1-135, 1-119/122 and full length alpha synuclein. The 9E4 does not affect the 1-119/122 species but only IPs full length and 1-135 (**Example 4**).

5 **[00038] Fig. 10** shows schematics of the proteolysis of alpha-synuclein fibrils cleaved by calpain at amino acid 119/122. Alpha-synuclein fibrils (PFF) are added to the culture with (PFF+) or without (PFF-) test antibody. The presence of GM-37/285 inhibit the formation of the truncated alpha-synuclein in cells and secreted into the cell media.

10 **[00039] Fig. 11A** shows that GM37 inhibits the formation of the truncated band (12KD) in both the media and in cell lysates of primary mouse cortical cultures treated with PFFs. Proteins were separated by SDS-PAGE and western blotted to detect different species of alpha-synuclein. In cells treated only with PFF or the control antibody (B12) two monomeric alpha-synuclein bands are detected at 12 and 14 kDa, representing truncated and full length alpha-synuclein, respectively. In the presence of GM-37 there is only a faint band at 12Kd indicating that the majority of the cleavage is blocked. This effect is also reflected in the media of the cells. The relative levels of accumulation may also be inhibited by the presence of GM-37 as reflected in the reduction in the relative intensity of the 14Kd band. Alternatively there may be reduced amount of the 14Kd band available for uptake by the cells. (**Example 5**).

20 **[00040] Fig. 11B** shows dose dependent inhibition of proteolysis of alpha-synuclein fibrils by antibodies GM37, GM37 variant 2 and GM285. In cell lysates from primary mouse cortical cultures at low antibody concentration (0,1 ug/ml) there are both a band representing full length (FL) alpha-synuclein and a band representing C-terminally truncated (CT) alpha-synuclein (indicated by arrows). Increasing antibody concentration to 1, 5 and 10 ug/ml leads to reduced proteolysis of alpha-synuclein fibrils in cells. This is observed with both antibody GM37, GM37v2 and GM285. Control samples are treated with a human IgG1 antibody B12 not recognising alpha-synuclein. There is also a control with no antibody added (No ab), and cells with no alpha-synuclein fibrils added (No Asyn). The total amount of alpha-synuclein is also reduced in samples treated with 37, 37v2 and 285 compared to B12 or "no antibody" control, 25 indicative that all three antibodies reduce accumulation of alpha-synuclein in cells in concentration dependent manner. The actin band on the top of the gel shows equal loading of the samples (**Example 5**).

[00041] Fig. 12 shows the impact of GM37 and GM285 on seeding of alpha-synuclein aggregation and alpha-synuclein phosphorylation in mouse primary cortical neurons.

- 12A) Example of images of primary neurons stained for phosphorylated alpha-synuclein, which appears as spots or punctate staining in cells when the cells are seeded with either 1 ng of pure seeds or crude seeds of alpha-synuclein.
- 12B) Western blot of proteins from primary cortical neurons separated in soluble and insoluble fractions. The blots were stained with human alpha-synuclein specific antibody (4B12/H a-syn), phospho-Ser-129-alpha-synuclein specific antibody (ab51253/pS-a-Syn) and mouse alpha-synuclein specific antibody (D37A2/M a-syn) and show that addition of the crude seeds in primary neurons leads to accumulation of endogenous mouse alpha-synuclein and phosphorylated alpha-synuclein and higher molecular weight multimers of alpha-synuclein in the insoluble fraction.
- 12C) GM37, GM37 variant 2 and GM285 inhibit appearance of phosphorylated alpha-synuclein quantitated as the number of alpha-synuclein phosphoserine 129 positive spots in cells by a Cellomics ARRAYSCAN™ automated microscope. GM37, GM37v2 and GM285 reduce the amount of phosphorylated alpha-synuclein spots in cells in dose dependent manner.
- 12D) Western blot of the homogenates from primary cortical neurons treated at the highest dose of antibody (133 nM), and stained for actin, human alpha-synuclein, phosphorylated alpha-synuclein and mouse alpha-synuclein shows that antibodies 37, 37v2 and 285 inhibit truncation of the alpha-synuclein crude seeds taken up by the cells in the insoluble fraction. All antibodies also inhibit the accumulation of phosphorylated, endogenous mouse and higher molecular weight multimers of phosphorylated mouse alpha-synuclein in the insoluble fraction. The actin band on the top of the gel shows equal loading of the samples (**Example 6**).

[00042] Fig 13 shows basal synaptic transmission at the Schaffer collateral-CA1 synapse in the hippocampus of F28-sncA transgenic and age-matched control mice.

- Field excitatory post-synaptic potentials (fEPSPs) were evoked by a single stimulus applied to the Schaffer collateral, and basal synaptic transmission was assessed by measuring the fEPSP slope as a function of the stimulation intensity. Short-term synaptic plasticity was evaluated by induction of paired-pulse facilitation. The different

intensities of stimulation were 0, 25, 50, 75, 100, 150, 200, 300, 400, and 500 μ A, and were applied successively in increasing order, with 2 to 3 repeats for each intensity. Basal synaptic transmission was found to be significantly impaired in F28-snca transgenic mice overexpressing wild-type alpha-synuclein compared to age-matched control mice (**Example 7**).

[00043] Fig 14 shows the effect of the systemic administration of a single dose of human 9E4 (15 mg/kg, i.p.) on the impairment in basal synaptic transmission at the Schaffer collateral- CA1 synapse in the hippocampus of F28-snca transgenic mice. Field excitatory post-synaptic potentials (fEPSPs) were evoked by a single stimulus applied to the Schaffer collateral, and basal synaptic transmission was assessed by measuring the fEPSP slope as a function of the stimulation intensity. Acute treatment with h9E4 induced a significant reversal of the impairment in basal synaptic transmission in F28-snca transgenic mice (Tg-snca + h9E4 vs. Tg-snca + PBS, $p=0.002$). However, the reversal by h9E4 was only partial, as indicated by a significantly lower basal synaptic transmission compared to littermates treated with PBS ($p=0.007$) (**Example 7**).

[00044] Fig 15 shows the effect of the systemic administration of a single dose of human GM37 (15 mg/kg, i.p) or an isotype control antibody (B12) on the impairment in basal synaptic transmission at the Schaffer collateral-CA1 synapse in the hippocampus of F28-snca transgenic mice. Field excitatory post-synaptic potentials (fEPSPs) were evoked by a single stimulus applied to the Schaffer collateral, and basal synaptic transmission was assessed by measuring the fEPSP slope as a function of the stimulation intensity. Acute treatment with GM37 induced full reversal of the impairment in basal synaptic transmission in F28-snca transgenic mice (Tg-snca + GM37 vs. Tg-snca + B12, $p=0.004$) (**Example 7**).

[00045] Fig 16 shows the effect of the systemic administration of a single dose of human GM285 (15 mg/kg, i.p) on the impairments in basal synaptic transmission at the Schaffer collateral-CA1 synapse in the hippocampus of F28-snca transgenic mice. Field excitatory post-synaptic potentials (fEPSPs) were evoked by a single stimulus applied to the Schaffer collateral, and basal synaptic transmission was assessed by measuring the fEPSP slope as a function of the stimulation intensity. Acute treatment with GM285 induced full reversal of the impairment in basal synaptic transmission in

F28-snca transgenic mice (Tg-snca + GM285 vs. Tg-snca + PBS, $p=0.001$) (**Example 7**).

[00046] **Fig 17 (Panels A-B)** shows the effect of the systemic administration (15 mg/kg, i.p.) of human 9E4, GM37 or isotype control antibody (anti-HEL) on the levels of human alpha-synuclein in the interstitial fluid (isf) in the hippocampus of freely moving F28-snca transgenic mice. The average of the two-three basal values (4h-6h) prior to antibody treatment was taken as baseline and set to 100% for each animal. Differences were analyzed using a two-way analysis of variance (ANOVA) with repeated measures. The basal levels of human alpha-synuclein in hippocampus were 8.1 ± 1.1 ng/ml (mean $\pm$ SEM, $n = 25$, not corrected for the in vitro dialysis probe recovery). The administration of GM37 induced a larger reduction in human alpha-synuclein in the hippocampus of F28 mice compared to both the comparator antibody, human 9E4, and the control isotype, anti-HEL. Timepoints that show significant differences in the levels of alpha-synuclein between animals treated with GM37 or the control antibody are indicated by an asterisk. (**Example 8**).

[00047] **Fig 18** shows a schematic representation of the timeline for antibody treatment (down arrows), viral injections and behavioural assessment in the rat AAV human alpha-synuclein model shown in **Fig. 19 (Example 9)**.

[00048] **Fig 19** shows that antibody GM37 can reduce Parkinsonian motor deficits after chronic treatment in the rat AAV model. The effect of chronic treatment with GM37 or PBS in AAV-human- alpha-synuclein rats on motor asymmetry is assessed in the cylinder test. Each rat was tested for the use of the forepaws by monitoring for 5 minutes. The percentage of use of the right forepaw (ipsilateral to the injection) and use of left (contralateral+ right forepaws) was calculated for each animal (as shown on the y-axis) *, ** $p<0.05$ and 0.01 compared to GFP-PBS rats. The rats treated with PBS still have a significant asymmetry in paw use, while animals treated with antibody GM37 have no longer a significant deficit. (**Example 9**).

[00049] **Fig 20A-20C** shows that chronic treatment with antibody GM37 can reduce pathological alpha-synuclein phosphorylation induced by injection of pathological alpha-synuclein fibrillary seeds into the mouse striatum. Fig 20A shows a schematic indicating relative treatment times with respect to seed injection and cell counting. The

antibody GM37 was administered one day before injection of recombinant alpha-synuclein fibrillary seeds into dorsal striatum of mice, and then weekly for six weeks. Dosing regimen was either 15 mg/kg iv or 30 mg/kg ip. Fig 20B shows the exposure level of GM37 in plasma based on site of injection and dose. Weekly samples were taken before the injection of new antibody dose. Fig 20C shows the exposure level of GM37 in csf based on dose and injection site at the end of the study. Fig 20D compares the number of cells with phosphorylated alpha-synuclein positive inclusions counted from every sixth section in substantia nigra after treatment with GM37 or PBS control. The mice treated with GM37 both 15 mg/kg iv and 30 mg/kg ip had a significant reduction in cells with phosphorylated alpha-synuclein inclusions compared to the PBS treated mice (**Example 10**).

[00050] Fig 21 shows alignment of human α (**SEQ ID NO:10**), β (**SEQ ID NO:37**) and γ (**SEQ ID NO:38**) synuclein proteins. Amino acid residues different from α -synuclein are highlighted. Gaps are indicated by a dot. SwissProt numbers are in parenthesis.

[00051] Fig 22 shows alignment of alpha-synuclein orthologs (Cynomolgus monkey, **SEQ ID NO:39**; Rat, **SEQ ID NO:40**; Mouse, **SEQ ID NO:41**). Amino acid residues different from human alpha-synuclein (**SEQ ID NO:10**) are highlighted. SwissProt numbers are shown in parenthesis.

[00052] Fig 23 shows transient expression of GM37 (named GM37 wild type (wt) and 3 GM37 variants, named GM37 var 1, 2 and 3. Asterisk indicates that the data are determined post protein A purification and neutralisation. † indicates that data are calculated from yield achieved post protein A and neutralization in relation to scale of expression culture (0.4L).

[00053] Fig 24 shows a competition ELISA measuring binding of four antibodies GM37 wt, GM37 var 1, GM37 var 2 and GM37 var 3 to human alpha-synuclein. Plates coated with alpha-synuclein are used to detect the amount of antibody remaining after preincubation in solution of each antibody (0.3 μ g/ml) with increasing concentration of alpha-synuclein (0-1000nM). All four antibodies show similar binding to alpha-synuclein.

[00054] Fig 25 shows a table comparing the binding rate kinetic parameters of GM37wt 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).

[00055] Fig. 26 compares the effect of alpha-synuclein antibodies on phosphorylated alpha-synuclein levels in murine primary neurons treated with pathological alpha-synuclein fibrillary seeds. Primary neurons were treated with seeds (10ng) in the presence or absence of four GM37, GM37 var 1, GM37 var 2 and GM37 var 3 (2µg). Neurons were fixed & stained after 3 weeks and analysed by Cellomics ARRAYSCAN™ for alpha-synuclein phospho serine 129 positive spots. Cells treated with seeds alone or with seeds plus the isotype control antibody (B12) show significantly increased levels phosphorylation. Cells treated with GM37wt and the 3 variants are able to inhibit phosphorylation of alpha-synuclein, they all show the same level of phosphorylation as cells that did not receive seeds. Data is shown as mean ±SD as determined from seven images per well in five wells. N=2.

[00056] Fig 27 compares temperature dependent aggregation of wt GM37, var1, var2 and var3. A sample of each of the antibodies was subjected to a steady increase in temperature over time and the level of aggregation was simultaneously measured by multi-angle light scattering (Prometheus NT.48, NanoTemper Technologies). The temperature for onset of aggregation is similar for GM37 and GM37-variants, however the lowest level of aggregation observed for GM37-Var2.

20 DETAILED DESCRIPTION OF THE INVENTION

Definitions

[00057] 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 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 E QVTNVGGAV VTGVTAVAQK
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TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE **GILEDMP**VDP
DNEAYEMPSE EGYQDYEP EA

[00058] The present invention relates to antibodies and to fragments of antibodies that are capable of specifically binding to alpha-synuclein, and in particular to human alpha-synuclein. In particular, the antibodies and fragments thereof exhibit the ability to specifically bind to an epitope within 112-117 of human alpha-synuclein.

[00059] 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 domain (abbreviated herein as VH) and a heavy chain constant domain, usually comprised of three domains (CH1, CH2 and CH3). Heavy chains can be of any isotype, including IgG (IgG1, IgG2, IgG3 and IgG4 subtypes), IgA (IgA1 and IgA2 subtypes), IgM and IgE. Each light chain is comprised of a light chain variable domain (abbreviated herein as VL) and a light chain constant domain (CL). Light chains include kappa chains and lambda chains. The heavy and light chain variable domain is typically responsible for antigen recognition, while the heavy and light chain constant domain 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 domains of the heavy and light chains contain a binding domain that interacts with an antigen. Of particular relevance are antibodies and their antigen-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.

[00060] The term "epitope" means an antigenic determinant capable of specific binding to an antibody. Epitopes usually consist of surface groupings of molecules such

as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and linear epitopes are distinguished in that the binding to the former, but not the latter, is always lost in the presence of denaturing solvents. The epitope may comprise amino acid residues directly involved in the binding and other amino acid residues, which are not directly involved in 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). The term “112-117 epitope” refers to a region of human alpha-synuclein that contains at least 4 of the 6 amino acid residues of 112-117 human alpha-synuclein, which epitope does not include any residue from 1-111 (including any residue from 106-111) of human alpha-synuclein, nor any residue from 118-140 (including residue 118-120) of human alpha-synuclein. As used herein, an antibody is said to be capable of specifically binding to an epitope within the “112-117 epitope” if it is capable of specifically binding to human alpha-synuclein by binding to at least 4 of the 6 amino acid residues of the 112-117 epitope.

[00061] As used herein, the term “antigen-binding fragment of an antibody” means a fragment, portion, region or domain of an antibody (regardless of how it is produced (*e.g.*, via cleavage, recombinantly, synthetically, *etc.*)) that is capable of specifically binding to an epitope, and thus the term “antigen-binding” is intended to mean the same as “epitope-binding” so that, for example, an “antigen-binding fragment of an antibody” is intended to be the same as an “epitope-binding fragment of an antibody”. An antigen-binding fragment may contain 1, 2, 3, 4, 5 or all 6 of the CDR Domains of such antibody and, although capable of specifically binding to such epitope, may exhibit a specificity, affinity or selectivity toward such epitope that differs from that of such antibody. Preferably, however, an antigen-binding fragment will contain all 6 of the CDR Domains of such antibody. An antigen-binding fragment of an antibody may be part of, or comprise, a single polypeptide chain (*e.g.*, an scFv), or may be part of, or comprise, two or more polypeptide chains, each having an amino-terminus and a carboxyl terminus (*e.g.*, a diabody, a Fab fragment, a Fab₂ fragment, *etc.*). Fragments of antibodies that exhibit antigen-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 naturally encoded by separate genes, or

polynucleotides that encode such gene sequences (*e.g.*, 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 antigen-binding molecules (known as single-chain Fv (scFv); see *e.g.*, Bird *et al.*,
5 (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 antigen-
10 binding molecule) (see for instance PNAS USA 90(14), 6444-8 (1993) for a description of diabodies). Examples of antigen-binding fragments encompassed within the present invention include (i) a Fab' or Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains, or a monovalent antibody as described in WO2007059782; (ii) F(ab')₂ fragments, bivalent fragments comprising two Fab
15 fragments linked by a disulfide bridge at the hinge domain; (iii) an Fd fragment consisting essentially of the VH and CH1 domains; (iv) a Fv fragment 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
20 nanobodies (Revets *et al.*; Expert Opin Biol Ther. 2005 Jan;5(1):11-24) and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, 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
25 molecules (known as single chain antibodies or single chain Fv (scFv), see 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
30 chimeric antibodies and humanized 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 or IgG4)

that is encoded by heavy chain constant domain 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.

5 **[00062]** The term “bispecific antibody” refers to an antibody containing two independent antigen-binding fragments that each target independent targets. These targets can be epitopes present on different proteins or different epitopes present on the same target. Bispecific antibody molecules can be made using compensatory amino acid changes in the constant domains of the HCs of the parent monospecific
10 bivalent antibody molecules. The resulting heterodimeric antibody contains one Fabs contributed from two different parent monospecific antibodies. Amino acid changes in the Fc domain leads to increased stability of the heterodimeric antibody with bispecificity that is stable over time. (Ridgway et al., Protein Engineering 9, 617-621 (1996), Gunasekaran et al., JBC 285, 19637-1(2010), Moore et al., MAbs 3:6 546-557
15 (2011), Strop et al., JMB 420, 204-219 (2012), Metz et al., Protein Engineering 25:10 571-580 (2012), Labrijn et al., PNAS 110:113, 5145 -5150 (2013), Spreter Von Kreudenstein et al., MAbs 5:5 646-654 (2013)). Bispecific antibodies can also include molecules that are generated using ScFv fusions. Two monospecific scfv are then independently joined to Fc domains able to form stable heterodimers to generate a
20 single bispecific molecule (Mabry et al., PEDS 23:3 115-127 (2010)). Bispecific molecules have dual binding capabilities. For example, targeting both a therapeutic target and a transcytosing surface receptor for the purpose of delivering a therapeutic antibody across the blood brain barrier to treat a CNS disease.

25 **[00063]** The terms GM37, GM-37, GM37 wild type (wt), mab37 and 6004-37 are used interchangeably herein and all refer to the same antibody.

[00064] The term antibody GM37 is intended to include 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**. In one embodiment, the antibody GM37 or antigen-binding fragment thereof may comprise or
30 consist of the heavy chain variable domain of **SEQ ID NO:7** and/or the light chain variable domain of **SEQ ID NO:8**. For example, the antibody GM37 may be an IgG antibody comprising a heavy chain consisting of a variable domain of **SEQ ID NO:7**

and a constant domain of **SEQ ID NO:18** together with a light chain consisting of a variable domain of **SEQ ID NO:8** and a kappa constant domain of **SEQ ID NO:17**.

[00065] Deamination of proteins, and in these instance antibodies, can occur spontaneously during manufacturing and storage, but also in vivo, and makes the quality of the final pharmaceutical medicament difficult to control. The deamination may also in some instances affect the activity of the molecule. Deamination occurs at asparagine residues, but the location of the relevant asparagine may be difficult to predict with certainty, but may be influenced in some instances by an asparagine-glycine motif. Several possible deamination motifs are found on the GM37 antibody, however, one likely site of deamination was found to be at residue 54 of the heavy chain. The subsequent substitution of asparagine by another amino acid is not straight forward, but 3 variants of GM37 (GM37 variant (var) 1, 2 and 3) were found to retain the activity of the original GM37 (GM37 wild type (wt)).

[00066] The term GM37 variants refers to the deaminated variants 1,2 or 3, wherein variant 1 has a N54S substitution, variant 2 has a N54Q substitution and variant 3 has a N54H compared to the GM37 antibody described herein above.

[00067] The antibody GM37 variant (var) 1, 2 and 3 are thus intended to include an antibody or antigen-binding fragment thereof comprising or consisting of the Heavy Chain as given in CDR1 and 3 **SEQ ID Nos:1 and 3** from GM 37 and the Light Chain CDR1-3 from GM37 as given in **SEQ ID Nos:4-6**, but differing in their heavy chain CDR2 so that variant 1 has CDR 2 of **SEQ ID NO:33**, variant 2 has CDR 2 of **SEQ ID NO:34** and variant 3 has CDR 2 of **SEQ ID NO:35**.

[00068] In one embodiment, the antibody GM37 variants or their antigen-binding fragments may comprise or consist of the heavy chain variable domain of **SEQ ID NO:30**, 31 and 32 for variant 1, 2 and 3, respectively, and the light chain variable domain of **SEQ ID NO:8**. The antibody GM37 may be an IgG antibody comprising a heavy chain consisting of a variable domain of **SEQ ID NO:30**, 31 or 32 and a constant domain of **SEQ ID NO:18** together with a light chain consisting of a variable domain of **SEQ ID NO:8** and a kappa constant domain of **SEQ ID NO:17**.

[00069] The terms GM285, GM-285, mab285 and 6004-285 are used interchangeably herein and all refer to the same antibody.

[00070] The term antibody GM285 is intended to include 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**. In one embodiment, the antibody GM37 or antigen-binding fragment thereof may
5 comprise or consist of the heavy chain variable domain of **SEQ ID NO:26** and/or the light chain variable domain of **SEQ ID NO:27**. For example, the antibody GM37 may be an IgG antibody comprising a heavy chain consisting of a variable domain of **SEQ ID NO:26** and a constant domain of **SEQ ID NO:28** together with a light chain consisting of a variable domain of **SEQ ID NO:27** and a kappa constant domain of **SEQ**
10 **ID NO:29**.

[00071] The GM285 antibody specifically binds an epitope within the sequence 112-115 (ILED; **SEQ ID NO:19**) of human alpha-synuclein (**SEQ ID NO:10**).

[00072] Unless otherwise specified herein, the numbering of amino acid residues in this region is according to IMGT®, the international ImMunoGeneTics information
15 system® or, Kabat, E. A., Wu, T. T., Perry, H. M., Gottesmann, K. S. & Foeller, C. (1991). Sequences of Proteins of Immunological Interest, 5th edit., NIH Publication no. 91-3242 U.S. Department of Health and Human Services. Chothia, C. & Lesk, A. M. (1987). Canonical structures for the hypervariable domains of immunoglobulins. J. Mol. Biol. 196, 901-917).

20 [00073] An "anti-alpha-synuclein antibody" or "alpha-synuclein antibody" (used interchangeably herein, depending on the context wherein its written) is an antibody or an antigen-binding fragment thereof which binds specifically to alpha-synuclein or an alpha-synuclein fragment as defined herein above, in particular the sequence of alpha-synuclein corresponding to SEQ ID NOs 9 and/or 19.

25 [00074] The term "human antibody" (which may be abbreviated to "humAb" or "HuMab"), as used herein, is intended to include antibodies having variable and constant domains 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
30 site-specific mutagenesis in vitro or during gene rearrangement or by somatic mutation in vivo).

The term "combined" as used herein in the context of the method/use of the invention comprises the administration of therapeutically effective amounts of 1) an alpha-synuclein antibody according to the invention and 2) a compound useful for the treatment of of Parkinson's disease (including idiopathic and inherited forms of Parkinson's disease), Gauchers Disease (GD), Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson's disease, pure autonomic failure and multiple system atrophy. The administration of the 2 medicaments may occur simultaneously or separately in therapeutically effective amounts to achieve the desired treatment effect of the combined treatment.

- 10 **[00075]** These additional medicaments may be selected from the group comprising dopamine, caspase inhibitors, calpain inhibitors, LRRK2 inhibitors, BACE1 inhibitors, aggregation inhibitors, antibodies recognising any form of Amyloid-beta, inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia, nicotine, caffeine, modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A2A antagonists to said subject in an effective amount.

- [00076]** According to an embodiment of the invention the additional medicaments are selected from the group comprising pramipexole, VX-765 (Pralnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, C16H15NO₄, BMS-986016, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.

- 25 **[00077]** The compounds used in the combinational treatment may be administered simultaneously or with a time gap between the administrations of the two compounds. The two compounds may be administered on the same day or on different days or time intervals.

- 30 **[00078]** The terms "monoclonal antibody" or "monoclonal antibody composition" as used herein refer to a preparation of antibody molecules of single molecular composition. A conventional monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. In certain embodiments a monoclonal

antibody can be composed of more than one Fab domain thereby increasing the specificity to more than one target. The terms "monoclonal antibody" or "monoclonal antibody composition" are not intended to be limited by any particular method of production (e.g., recombinant, transgenic, hybridoma, etc.).

- 5 **[00079]** The term "humanized" refer to a molecule, generally prepared using recombinant techniques, having an antigen-binding site derived from an immunoglobulin from a non-human species 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
10 to human constant domains, or only the complementarity determining regions (CDRs) of such variable domains grafted to appropriate human framework regions of 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
15 basis for humanization. Humanization lessens or eliminates the likelihood that a constant domain of the molecule will act as an immunogen in human individuals, but the possibility of an immune response to the foreign variable domain 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).
- 20 Another approach focuses not only on providing human-derived constant domains, but modifying the variable domains as well so as to reshape them as closely as possible to human form. It is known that the variable domains 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
25 regions (FRs) which are relatively conserved in a given species and which putatively provide a scaffolding for the CDRs. When nonhuman antibodies are prepared with respect to a particular antigen, the variable domains 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
30 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; Verhoeyen, 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).
- 20 **[00080]** As used herein, an antibody or an antigen-binding fragment thereof is said to “specifically” 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. In one embodiment, the antibody, or antigen-binding fragment thereof, of the invention binds at least 10-fold
- 25 more strongly to its target (human alpha synuclein) than to another molecule; preferably at least 50-fold more strongly and more preferably at least 100-fold more strongly. Preferably, the antibody, or antigen-binding fragment thereof, binds under physiological conditions, for example, *in vivo*. Thus, an antibody that is capable of “specifically binding” to an epitope within residues 112-117 (ILEDMP (**SEQ ID NO:9**))
- 30 of human alpha-synuclein encompasses an antibody or antigen-binding fragments thereof, that is capable of binding to an epitope within residues 112-117 of human alpha-synuclein with such specificity and/or under such conditions. Methods suitable for determining such binding will be known to those skilled in the art, and exemplary

methods are described in the accompanying Examples. 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 either a BIAcore® 3000 or T200 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.

[00081] The term "kd" (sec⁻¹ or 1/s), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. Said value is also referred to as the koff value.

[00082] The term "ka" (M⁻¹ x sec⁻¹ or 1/Msec), as used herein, refers to the association rate constant of a particular antibody-antigen interaction.

[00083] The term "KD" (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the kd by the ka.

[00084] The term "KA" (M⁻¹ or 1/M), as used herein, refers to the association equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the ka by the kd.

[00085] In one embodiment, the invention relates to an antibody or antigen-binding fragments thereof, which exhibits one or more of the following properties:

- i. a binding affinity (KD) for alpha-synuclein of between 0.5-10 nM, such as 1-5 nM or 1-2 nM;
- ii. capability of inhibiting protease truncation of alpha-synuclein fibrils;

- iii. capability of reversing impairment in basal synaptic transmission in F28-snca transgenic mice;
- iv. capability of reducing levels of alpha-synuclein in the mouse hippocampus as measured by in vivo microdialysis;
- 5 v. capability, when administered chronically, to restore motor function in a rat model of Parkinson's disease
- vi. Capability to prevent seeding of alpha-synuclein (such as accumulation of insoluble phosphorylated alpha-synuclein in vitro and/or in a mouse model of Parkinson's disease); and/or
- 10 vii. Capability to bind truncated alpha-synuclein in a human brain.

[00086] The binding affinity (KD) for alpha-synuclein may be determined using methods well known in the art, *e.g.* as described in Example 2.

[00087] The term "capability of inhibiting protease truncation of alpha-synuclein fibrils" includes the capability of inhibiting calpain-1 induced formation of fragment 1-119-122
15 of human alpha synuclein in primary cortical neurons (see **Example 5**).

[00088] The term "capability of reversing impairment in basal synaptic transmission in F28-snca transgenic mice" includes the capability of reverse the impairment of synaptic transmission and plasticity in the CA1 area of the hippocampus in F28-snca transgenic mice, for example as indicated by evoked fEPSP slope as measured
20 electrophysiologically (See **Example 6**).

[00089] The term "capability of reducing levels of alpha-synuclein in the mouse hippocampus as measured by in vivo microdialysis" includes the capability of reducing levels of human alpha synuclein in the hippocampus awake, freely-moving F28-snca transgenic mice, as measured using in vivo microdialysis (see **Example 7**).

25 **[00090]** The term "capability, when administered chronically, to restore motor function in a rat model of Parkinson's disease" include the capability to reduce or eliminate motor asymmetry in a rat recombinant adeno-associated viral vector (rAAV) model of Parkinson's Disease (see **Example 8**).

[00091] In some antibodies, only part of a CDR, namely the subset of CDR residues
30 required for binding, termed the SDRs, are needed to retain binding in a humanized

antibody. CDR residues not contacting the relevant epitope 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 domains 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 CDR 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.

[00092] The fact that a single amino acid alteration of a CDR residue can result in loss of functional binding (Rudikoff, S. *etc.* (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.iiij 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.ijj). **Table 1** presents the BLOSUM62.ijj 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 alpha-synuclein, for example, then the BLOSUM62.ijj 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.ijj 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

[00093] 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:

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

5

[00094] More conservative substitutions groupings include: valine-leucine-isoleucine, phenylalanine-tyrosine, lysine-arginine, alanine-valine, and asparagine-glutamine.

[00095] 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

[00096] 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 labeled 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).

[00097] 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 Human Monoclonal Antibody Against HIV-1 gp41,” *MAbs* 1(5):462-474; Gustchina, E. *et al.* (2009) “Affinity Maturation By Targeted Diversification Of The CDR-H2 Loop Of A Monoclonal Fab Derived From A Synthetic Naïve Human Antibody Library And Directed Against The Internal Trimeric Coiled-Coil Of Gp41 Yields A Set Of Fabs With Improved HIV-1 Neutralization Potency And Breadth,” *Virology* 393(1):112-119; Finlay, W.J. *et al.* (2009) “Affinity Maturation Of A Humanized Rat Antibody For Anti-RAGE Therapy: Comprehensive Mutagenesis Reveals A High Level Of Mutational Plasticity Both Inside And Outside The Complementarity-Determining Regions,” *J. Mol. Biol.* 388(3):541-558; Bostrom, J. *et al.* (2009) “Improving Antibody Binding Affinity And Specificity For Therapeutic Development,” *Methods Mol. Biol.* 525:353-376; Steidl, S.

et al. (2008) "In Vitro Affinity Maturation Of Human GM-CSF Antibodies By Targeted CDR-Diversification," *Mol. Immunol.* 46(1):135-144; and Barderas, R. *et al.* (2008) "Affinity Maturation Of Antibodies Assisted By In Silico Modeling," *Proc. Natl. Acad. Sci. (USA)* 105(26):9029-9034.

5 **[00098]** Thus, the sequence of CDR variants of encompassed antibodies or their antigen-binding fragments may differ from the sequence of the CDR of the parent antibody, GM37, GM37 var 1-3, or 285, through substitutions; for instance substituted 4 amino acid residue, 3 amino acid residue, 2 amino acid residue or 1 of the amino acid residues. According to an embodiment of the invention it is furthermore envisaged that
10 the amino acids in the CDR regions may be substituted with conservative substitutions, as defined in the 3 tables above.

[00099] The term "treatment" or "treating" as used herein means ameliorating, slowing, attenuating or reversing the progress or severity of a disease or disorder, or ameliorating, slowing, attenuating or reversing one or more symptoms or side effects of
15 such disease or disorder. For purposes of this invention, "treatment" or "treating" further means an approach for obtaining beneficial or desired clinical results, where "beneficial or desired clinical results" include, without limitation, alleviation of a symptom, diminishment of the extent of a disorder or disease, stabilized (i.e., not worsening) disease or disorder state, delay or slowing of the progression a disease or disorder
20 state, amelioration or palliation of a disease or disorder state, and remission of a disease or disorder, whether partial or total, detectable or undetectable.

[000100] An "effective amount," when applied to an antibody or antigen-binding fragments thereof, 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
25 therapeutic result including, without limitation, clinical results. The phrase "therapeutically effective amount" when applied to an antibody or antigen-binding fragments thereof, of the invention is intended to denote an amount of the antibody, or antigen-binding fragment thereof, that is sufficient to ameliorate, palliate, stabilize, reverse, slow, attenuate or delay the progression of a disorder or disease state, or of a
30 symptom of the disorder or disease. In an embodiment, the method of the present invention provides for administration of the antibody, or antigen-binding fragment thereof, in combinations with other compounds. In such instances, the "effective

amount” is the amount of the combination sufficient to cause the intended biological effect.

[000101] A therapeutically effective amount of an anti-alpha-synuclein antibody or antigen-binding fragment thereof of the invention 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.

[000102] As indicated above, the present invention particularly relates to a monoclonal antibody capable of specifically binding to an epitope within amino acids 112-117 of human alpha-synuclein (**SEQ ID NO:9** (ILEDMP)). In one embodiment the antibody is capable of competing with the antibody GM37 for binding to an epitope within the 112-117 amino acids of alpha-synuclein.

[000103] The antibodies of the present invention, exemplified by GM37 its variants GM37 var 1-3 and GM285, and their alpha-synuclein binding fragments are capable of binding the toxic alpha-synuclein fragment consisting of residues 1-119/122 of alpha-synuclein and neutralizing its toxicity (for example, by extracellular binding to the alpha-synuclein fragment and thereby preventing it from being taken up by cells. Surprisingly the antibodies of the present invention, which are capable of binding to an epitope within amino acids 112-117 of alpha-synuclein are superior to prior art antibodies such as antibody 9E4 in binding to toxic alpha-synuclein species in human brain, and have superior effects on clearing extracellular alpha-synuclein and normalising an impaired synaptic transmission induced by alpha-synuclein in vivo. The antibodies of the invention are also able to ameliorate the appearance of a relevant motor phenotype in a rat model for Parkinson's disease.

[000104] The antibodies of the present invention are preferably human or humanized antibodies.

[000105] The present invention also provides a method of reducing alpha-synuclein aggregate formation in a patient, comprising administering to the patient in need of such treatment, a therapeutically effective amount of an antibody of the invention.

[000106] The antibodies may be administered together, either simultaneously or separately, with another medicament selected from the group comprising dopamine, caspase inhibitors, calpain inhibitors, LRRK2 inhibitors, BACE1 inhibitors, aggregation inhibitors, antibodies recognising any form of Amyloid-beta, inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia, nicotine, caffeine, modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A₂A antagonists.

[000107] These medicaments may specifically be selected from the group comprising pramipexole, VX-765 (Prlnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzumab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, C16H15NO₄, BMS-986016, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.

[000108] 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 synucleinopathies such as Parkinson's disease (including idiopathic inherited forms of Parkinson's disease), Gaucher's Disease, Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson disease, pure autonomic failure and multiple system atrophy.

[000109] The treatment envisioned by the present invention 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.

[000110] The antibodies of antigen-binding fragments thereof of the present invention may be produced in different cell lines, such as a human cell line, a mammal non-human cell line, and insect cell line, for example a CHO cell line, HEK cell line, BHK-21 cell line, murine cell line (such as a myeloma cell line), fibrosarcoma cell line, PER.C6 cell line, HKB-11 cell line, CAP cell line and HuH-7 human cell line (Dumont et

al, 2015, Crit Rev Biotechnol. Sep 18:1-13., the contents which is included herein by reference).

[000111] The antibodies of the present invention may for example be monoclonal antibodies produced by the hybridoma method first described by Kohler et al., Nature 5 256, 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 10 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 non-human mammals such as rats, rabbits, dogs, 15 sheep, goats, primates, etc.

[000112] 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 20 include mice referred to herein as HuMAb mice and KM mice, respectively.

[000113] 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 (κ) chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous μ and κ chain loci (Lonberg, N. et al., Nature 25 368, 856-859 (1994)). Accordingly, the 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, 30 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), Tuaille 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, 5 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.

[000114] The HCo7, HCo12, HCo17 and HCo20 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen et al., EMBO J. 12, 10 811-820 (1993)), a CMD disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), and a KCo5 human kappa light chain transgene (as described in Fishwild et al., Nature Biotechnology 14, 845-851 (1996)). Additionally, the HCo7 mice have a HCo7 human heavy chain transgene (as described in US 5,770,429), the HCo12 mice have a HCo12 human heavy chain transgene (as 15 described in Example 2 of WO 01/14424), the HCo17 mice have a HCo17 human heavy chain transgene (as described in Example 2 of WO 01/09187) and the HCo20 mice have a HCo20 human heavy chain transgene. The resulting mice express human immunoglobulin heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

[000115] 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 25 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. HCo12-Balb/c, HCo17-Balb/c and HCo20-Balb/c mice can be generated by crossing HCo12, HCo17 and HCo20 to KCo5[J/K](Balb) as described in WO 09/097006.

[000116] 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 trans-chromosome composed of chromosome 14 antigen-binding fragment hCF
5 (SC20) as described in WO 02/43478.

[000117] Splenocytes from these transgenic mice may be used to generate hybridomas that secrete human monoclonal antibodies according to well-known techniques. Human monoclonal or polyclonal antibodies of the present invention, or antibodies of the present invention originating from other species may also be
10 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
15 5,827,690, US 5,756,687, US 5,750,172 and US 5,741,957.

[000118] 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 domains, kappa or lambda, may be used. If desired, the class of an
20 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
25 may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3 or IgG4 antibody for various therapeutic uses. In one embodiment an antibody of the present invention is an IgG1 antibody, for instance an IgG1, κ . An antibody is said to be of a particular isotype if its amino acid sequence is most homologous to that isotype, relative to other isotypes.

30 **[000119]** 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.

[000120] Antibodies and antigen-binding fragments thereof may e.g. be obtained by antigen-binding fragmentation using conventional techniques, and antigen-binding fragments screened for utility in the same manner as described herein for whole antibodies. For example, F(ab')₂ antigen-binding fragments may be generated by treating antibody with pepsin. The resulting F(ab')₂ antigen-binding fragment may be treated to reduce disulfide bridges to produce Fab' antigen-binding fragments. Fab' antigen-binding fragments may be obtained by treating an IgG antibody with papain; Fab' antigen-binding fragments may be obtained with pepsin digestion of IgG antibody. An F(ab') antigen-binding fragment may also be produced by binding Fab'-described below via a thioether bond or a disulfide bond. A Fab' antigen-binding fragment is an antibody antigen-binding fragment obtained by cutting a disulfide bond of the hinge domain of the F(ab')₂. A Fab'- antigen-binding fragment may be obtained by treating an F(ab')₂ antigen-binding fragment with a reducing agent, such as dithiothreitol. Antibody antigen-binding fragment may also be generated by expression of nucleic acids encoding such antigen-binding 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 antigen-binding fragment could include DNA sequences encoding the CH1 region and hinge domain of the H chain, followed by a translational stop codon to yield such a truncated antibody antigen-binding fragment molecule.

[000121] 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 domain. 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

5 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 domain and, as required by the Ig subtype, other regions of the CH region, such as the CH3

10 region, does not comprise 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

15 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).

[000122] Similarly, in one embodiment, the anti- alpha-synuclein antibody is a

20 monovalent antibody, which comprises:

(i) a variable domain of an antibody of the invention as described herein or an antigen-binding part of the said region, and

(ii) a CH domain of an immunoglobulin or a domain thereof comprising the CH2 and CH3 domains, wherein the CH domain or domain thereof has been modified such

25 that the domain corresponding to the hinge domain and, if the immunoglobulin is not an IgG4 subtype, other domains of the CH domain, such as the CH3 domain, do not comprise any amino acid residues, which are capable of forming disulfide bonds with an identical CH domain or other covalent or stable non-covalent inter-heavy chain bonds with an identical CH domain in the presence of polyclonal

30 human IgG.

[000123] In a further embodiment, the heavy chain of the monovalent anti-alpha-synuclein antibody has been modified such that the entire hinge domain has been deleted.

[000124] 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.

[000125] The invention also includes "Bispecific Antibodies," wherein an anti-
5 Alpha-synuclein binding region (*e.g.*, a Alpha-synuclein-binding region of an anti-alpha-synuclein monoclonal antibody) is part of a bivalent or polyvalent bispecific scaffold that targets more than one epitope, (for example a second epitope could comprise an epitope of an active transport receptor, such that the Bispecific Antibody would exhibit improved transcytosis across a biological barrier, such as the Blood Brain Barrier).
10 Thus, in another further embodiment, the monovalent Fab of an anti-synuclein antibody may be joined to an additional Fab or scfv that targets a different protein to generate a bispecific antibody. A bispecific antibody can have a dual function, for example a therapeutic function imparted by an anti-synuclein binding domain and a transport function that can bind to a receptor molecule to enhance transfer cross a biological
15 barrier, such as the blood brain barrier.

[000126] Anti-alpha-synuclein antibodies, and antigen-binding fragments thereof, 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
20 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
25 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.

30 [000127] The anti-alpha-synuclein antibodies and antigen-binding fragments thereof 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 the alpha-synuclein selectivity and/or the anti-alpha-synuclein specificity associated 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 (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 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 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.

[000128] 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).

[000129] The antibodies of antigen-binding fragments thereof of the present invention may be produced in different cell lines, such as a human cell line, a mammal non-human cell line, and insect cell line, for example a CHO cell line, HEK cell line, BHK-21 cell line, murine cell line (such as a myeloma cell line), fibrosarcoma cell line, PER.C6 cell line, HKB-11 cell line, CAP cell line and HuH-7 human cell line (Dumont et al, 2015, Crit Rev Biotechnol. Sep 18:1-13., the contents which is included herein by reference).

[000130] In an even further aspect, the invention relates to a recombinant eukaryotic or prokaryotic host cell, such as a transfectoma, which produces an antibody or an antigen-binding domain thereof of the invention as defined herein or a bispecific molecule of the invention as defined herein. Examples of host cells include yeast, bacteria, and mammalian cells, such as CHO or HEK cells. For example, in one embodiment, the present invention provides a cell comprising a nucleic acid stably integrated into the cellular genome that comprises a sequence coding for expression of an anti-alpha-synuclein antibody of the present invention or an antigen-binding fragment thereof. In another embodiment, the present invention provides a cell comprising a non-integrated nucleic acid, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a sequence coding for expression of an anti-alpha-synuclein antibody of the invention.

[000131] In one embodiment, the invention relates to a preparation that, as such term is used herein, comprises an anti-alpha-synuclein antibody as defined herein, and that is substantially free of naturally-arising antibodies that are either not capable of binding to alpha-synuclein or that do not materially alter the anti-alpha-synuclein functionality of the preparation. Thus, such a preparation does not encompass naturally-arising serum, or a purified derivative of such serum, that comprises a mixture of an anti-alpha-synuclein antibody and another antibody that does not alter the functionality of the anti-alpha-synuclein antibody of the preparation; wherein such functionality is selected from the group consisting of:

- (i) a binding affinity (KD) of the anti-alpha-synuclein antibody for alpha-synuclein;
- (ii) a capability of the anti-alpha-synuclein antibody of inhibiting protease truncation of alpha-synuclein fibrils;
- (iii) a capability of the anti-alpha-synuclein antibody of reversing impairment in basal synaptic transmission in F28-snca transgenic mice;
- (iv) a capability of the anti-alpha-synuclein antibody of reducing levels of alpha-synuclein in the mouse hippocampus as measured by in vivo microdialysis; and
- (v) a capability, when administered chronically, of the anti-alpha-synuclein antibody to restore motor function in a rat model of Parkinson's disease.

- (vi) a capability to prevent seeding of alpha-synuclein (such as accumulation of insoluble phosphorylated alpha-synuclein in vitro and/or in a mouse model of Parkinson's disease); and/or
- (vii) a capability to bind truncated alpha-synuclein in a human brain.

5

[000132] The invention particularly relates to preparations of such an anti-alpha-synuclein antibody having a structural change in its amino acid sequence (in any of its CDRs, variable domains, framework residues and/or constant domains) relative to the structure of a naturally-occurring anti-alpha-synuclein antibody, wherein said structural
10 change causes the anti-alpha-synuclein monoclonal antibody to exhibit a markedly altered functionality (i.e., more than a 20% difference, more than a 40% difference, more than a 60% difference, more than an 80% difference, more than a 100% difference, more than a 150% difference, more than a 2-fold difference, more than a 4-fold difference, more than a 5-fold difference, or more than a 10-fold difference in
15 functionality) relative to the functionality exhibited by said naturally-occurring anti-alpha-synuclein antibody; wherein such functionality is:

- (i) a binding affinity (KD) of the anti-alpha-synuclein monoclonal antibody for alpha-synuclein;
- (ii) a capability of the anti-alpha-synuclein monoclonal antibody of inhibiting
20 protease truncation of alpha-synuclein fibrils;
- (iii) a capability of the anti-alpha-synuclein monoclonal antibody of reversing impairment in basal synaptic transmission in F28-snca transgenic mice;
- (iv) a capability of the anti-alpha-synuclein monoclonal antibody of reducing
25 levels of alpha-synuclein in the mouse hippocampus as measured by in vivo microdialysis; and/or
- (v) a capability, when administered chronically, of the anti-alpha-synuclein monoclonal antibody to restore motor function in a rat model of Parkinson's disease;
- (vi) a capability to prevent seeding of alpha-synuclein (such as accumulation of
30 insoluble phosphorylated alpha-synuclein in vitro and/or in a mouse model of Parkinson's disease); and/or
- (vii) a capability to bind truncated alpha-synuclein in a human brain.

especially wherein such altered functionality is a result of the structural change and thus is inseparable from it.

5 **[000133]** The term “substantially free” of naturally-arising antibodies refers to the complete absence of such naturally-arising antibodies in such preparations, or of the inclusion of a concentration of such naturally-arising antibodies in such preparations that does not materially affect the alpha-synuclein-binding properties of the preparations. An antibody is said to be “isolated” if it has no naturally-arising counterpart or has been separated or purified from components which naturally accompany it.

10 **[000134]** The term “naturally-arising antibodies,” as it relates to such preparations, refers to antibodies (including naturally-arising autoantibodies) elicited within living humans or other animals, as a natural consequence to the functioning of their immune systems.

15 **[000135]** Thus, the preparations of the present invention do not exclude, and indeed explicitly encompass, such preparations that contain an anti-alpha-synuclein antibody and a deliberately added additional antibody capable of binding to an epitope that is not possessed by alpha-synuclein. Such preparations particularly include embodiments thereof wherein the preparation exhibits enhanced efficacy in treating synucleinopathies such as Parkinson's disease (including idiopathic and inherited form of Parkinson's disease), Gaucher's Disease, Diffuse Lewy Body Disease (DLBD), Lewy
20 body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson disease, pure autonomic failure and multiple system atrophy.

25 **[000136]** In an even further aspect, the invention relates to a pharmaceutical composition comprising:
(i) an anti-alpha-synuclein antibody or antigen-binding fragment thereof, both as defined herein or a preparation, as such term is defined herein, that comprises such an anti-alpha-synuclein antibody or antigen-binding fragment thereof, and
(ii) a pharmaceutically-acceptable carrier.

30 **[000137]** 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, 22nd Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 2013.

[000138] 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 epitope binding.

[000139] A pharmaceutical composition of the present invention may also include diluents, fillers, salts, buffers, detergents (e.g., a non-ionic 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).

[000140] 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.

[000141] 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. 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. In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

[000142] 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.

[000143] Examples of suitable aqueous and non-aqueous 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.

[000144] 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.

- 5 **[000145]** Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

- 10 **[000146]** 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
15 acid, and the like.

[000147] 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.

- 20 **[000148]** 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
25 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
30 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.

[000149] In one embodiment, the compounds of the present invention may be formulated to ensure proper distribution in vivo. Pharmaceutically acceptable carriers
5 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
10 present invention is contemplated. Supplementary active compounds may also be incorporated into the compositions.

[000150] 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, micro-emulsion, liposome, or other ordered structure suitable
15 to high drug concentration. The carrier may be an aqueous or non-aqueous 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
20 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 antibody
25 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
30 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.

[000151] 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.

[000152] Dosage regimens in the above methods of treatment and uses described herein 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.

[000153] 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.

[000154] A physician having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician 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 depend upon the factors described above. Administration may e.g. be intravenous, intramuscular, intraperitoneal, or subcutaneous. If desired, the effective daily dose of a pharmaceutical composition may be administered as two, three, four, 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.

[000155] In a further aspect, the invention relates to an antibody or antigen-binding fragments thereof, of the invention, for use in medicine.

[000156] In a further aspect, the invention relates to an antibody or antigen-binding fragments thereof, of the invention, for use in treating a synucleinopathy in combination with another medicament selected from the group comprising dopamine, caspase inhibitors, calpain inhibitors, LRRK2 inhibitors, BACE inhibitors, aggregation inhibitors, A β antibodies, inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia, nicotine, caffeine and modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A2A antagonists.

[000157] In one embodiment the medicament may be selected from the group comprising pramipexole, VX-765 (Pralnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, C16H15NO₄, BMS-986016,
 5 GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline

[000158] In one further embodiment, the monoclonal antibody, or antigen-binding fragment thereof, together with an additional medicament referred to herein above is for
 10 use in treating Parkinson's disease, idiopathic Parkinson's disease, familiar forms of Parkinson's Disease, Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson's disease, pure autonomic failure or multiple system atrophy.

[000159] In a further aspect, the invention relates to the use of the antibody, or
 15 antigen-binding fragment thereof, of the invention together with an additional medicament mentioned herein above, in the manufacture of a medicament for treating a synucleinopathy.

[000160] In another embodiment the invention relates to the use of an antibody of the invention for the preparation of a medicament for use in treatment of Parkinson's
 20 disease (including idiopathic and inherited forms of Parkinson's disease), Gauchers Disease (GD), Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson's disease, pure autonomic failure and multiple system atrophy wherein the patient is treated with an additional medicament selected from the group comprising dopamine, caspase inhibitors, calpain
 25 inhibitors, LRRK2 inhibitors, BACE inhibitors, aggregation inhibitors, antibodies against A β , inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia, nicotine, caffeine and modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A₂A antagonists. The patient may in a further embodiment be
 30 treated with an additional medicament selected from the group comprising pramipexole, VX-765 (Pralnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzemab, Solanezumab, Gantenerumab,

Crenezumab, Aducanumab, C16H15NO₄, BMS-986016, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.

[000161] In a further aspect, the invention relates to a treating Parkinson's disease or other synucleinopathies, comprising administering an effective dosage of an antibody of the invention, or an antigen-binding fragment thereof together with an effective therapeutic amount of another medicament mentioned herein above.

[000162] Preferably, in the uses and methods of those aspects of the invention, the treatment is chronic, and is preferably for at least 2 weeks, such as at least for 1 month, 6, months, 1 year or more.

[000163] Furthermore the invention relates to a kit comprising an antibody according to the invention and a medicaments selected from the group comprising dopamine, caspase inhibitors, calpain inhibitors, LRRK2 inhibitors, BACE inhibitors, aggregation inhibitors, antibodies against A β , inflammation inhibitors, LAG-3 antibodies, Isradipine, molecules reversing alpha-synuclein induced reduction in phagocytic activity of microglia, nicotine, caffeine and modulators of GCase, MAO-B inhibitors, Levodopa/carbidopa, Dopamine agonists, COMT inhibitors and A2A antagonists. The kit may in another embodiment contain an antibody according to the invention and a second medicament selected from the group comprising pramipexole, VX-765 (Prlnacasan), ABT-957, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, EGCG, Bapineuzumab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, C16H15NO₄, BMS-986016, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat (Cerdelga), Azilect, Eldepryl, Zelapar, Pramipexole, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefylin.

[000164] The kit may for the use in treating Parkinson's disease), Gauchers Disease (GD), Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson's disease, pure autonomic failure and multiple system atrophy.

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	GM285 CDR 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 HC
SEQ ID NO:43	9E4 LC

EXAMPLES

Example 1: Antibody Screening

1. Immunogen and ligand production

[000165] 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.

[000166] 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:

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MAHHHHHHIE GRMDVFMKGL SKAKEGVVAA AEKTKQGVAE AAGKTKEGVL
YVGSKTKEGV VHGVATVAEK TKEQVTNVGG AVVTGVTAVA QKTVEGAGSI
AAATGFVKKD QLGKNEEGAP QEGILEDMPV D (SEQ ID NO:16)
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was synthesized by Genscript and cloned into NdeI-XhoI site in pET24a(+) expression vector (Novagen).

[000167] 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.

[000168] 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.

[000169] 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.

[000170] 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.

[000171] 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.

[000172] 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 (fig 21 and 22) 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

[000173] 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.

[000174] 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.

[000175] 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.

[000176] The immunization protocols are shown in **Fig 1**.

[000177] Antibody 37 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.

[000178] Antibody 285 came from an immunization protocol where 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

[000179] HuMab mice with sufficient antigen-specific titer development as 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 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 μ M 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 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 cynomolgus 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 α -synuclein derivative that lacked residues 120-140 of α -synuclein.

[000180] The screening of anti- α -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.

[000181] 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.

[000182] 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 is 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.

[000183] 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 harvest medium. After seven days, the supernatants of the sub clones were 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).

[000184] 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.

- 5 **[000185]** The binding of 37 and 285 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

- 10 **[000186]** 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), according to the manufacturer's instructions. VH and VL coding regions were amplified by PCR and cloned directly, in frame, in the p33G1f and p33Kappa expression vectors (containing the human
15 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
20 FreestyleTM 293-F cells using 293fectin.

[000187] 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.

- 25 **[000188]** 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**)

5. Expression/Purification of Antibodies

- [000189]** Antibodies were produced by transfection in HEK293 6E cells using the
30 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, 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.

Example 2: Antibody Characterization Using Surface Plasmon Resonance

[000190] 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 25-3200RU, 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 samples were kept at 4 °C before acquisition.

[000191] 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** showing binding of antibody 37, 285 and 9E4 to human alpha-synuclein.

Example 3: Epitope Mapping

[000192] 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 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 (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 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 37 and 285 to peptides containing the sequence ILEDMP or ILED respectively can be seen in **Figure 7**.

Example 4: Immunoprecipitation of alpha-synuclein from human brain homogenates of cingulate cortex from patients with dementia with Lewy bodies

[000193] The ability of the antibodies to bind to and pull down alpha-synuclein from crude homogenates of cingulate cortex from human DLB or healthy control (marked with *) was analyzed by immunoprecipitation. Frozen sample from human cingulate cortex (obtained through Tissue Solutions Ltd, Scotland) was dissected in cryostat, and 100mg sample was added to 1600µl cellytic M cell lysis reagent (Sigma C2978) containing protease inhibitors and phosphatase inhibitors (Roche). Brain tissue was homogenized until the sample is dissolved completely using Precellys bead homogenizer (Bertin technologies, France) 4x30 sec at 5000 rpm. The solution was centrifuged at 3000 x g and the supernatant was used as the crude homogenate for immunoprecipitation.

[000194] For immunoprecipitation 10 µg of antibody was mixed with magnetic Dynabeads protein G beads using manufacturer's instructions (Life Technologies,

Paisley, UK). The crude brain homogenate was diluted 30 fold in lysis buffer (Sigma). Antibody coupled dynabeads were mixed with 500 ul of diluted homogenate and incubated 90 minutes at room temperature under continuous mixing in a rotator. After incubation the beads were washed in washing buffer and the bound antigens were

5 eluted using the non-denaturing elution buffer according to manufacturer's instructions (Dynabeads G protocol, Life Technologies, Paisley UK). The yield of the immunoprecipitation was visualized by Western blotting with detection mouse monoclonal anti-human alpha-synuclein antibody, (4B12, Thermo Scientific). The patterns of bands representing different molecular weight forms of alpha-synuclein

10 being pulled down differ between the 37, 37 variant 2 and 285 antibodies and the comparator antibody 9E4 in that the 37, 37v2 and 285 antibody can immunoprecipitate the major alpha-synuclein species, the full length alpha-synuclein (FL asyn 1-140) and the C-terminal terminal truncated species (1-135 and 1-119/122), while antibody 9E4 cannot immunoprecipitate the truncated species 1-119/122. **Figure 9.**

15 **Example 5: Inhibition of protease truncation of alpha-synuclein fibrils by antibodies in cell culture**

[000195] Recombinant alpha-synuclein monomers and fibrils can be taken up by primary neurons in culture. As shown schematically in Figure 10, after uptake of the alpha-synuclein in neurons, it can be processed by intracellular proteases, such as

20 Calpain I, with the major protease sensitive site at amino acid 119/122. To investigate truncation of alpha-synuclein by proteases mouse primary cortical neurons were prepared as described in Elvang et al. 2009 (Elvang et al.. J Neurochem. 2009;110(5):1377-87) and treated with cytarabine on DIV3 (3 days in vitro) to inhibit astrocyte growth. On DIV4 (4 days in vitro), the neurons were treated with sonicated (5

25 min at 50 % power in cup horn sonicator) pre-formed alpha-synuclein fibrils (PFFs) at an end concentration of 0.7 μ M alone or together with antibodies in the indicated concentrations. After 24 hours of incubation, the media was harvested and the cells were lysed. Western Blots was run on both media and cell lysate using the 4B12 antibody (Fig 11A) (Pierce MA1-90346) and a secondary anti-mouse antibody. After

30 probing with 4B12 + anti-mouse, the blots were stripped and reprobed with an anti-human-IgG antibody. On blot with 4B12, it can be seen that in the media from cells treated with PFFs only, there were strong bands at 14 and 12 kDa, where 14 kDa represents the full-length alpha-synuclein (FL-asyn) and 12 kDa represents the C-

terminally truncated fragment 1-119/122 (CT- α syn). In addition to that, there were higher molecular weight bands, most likely representing SDS-resistant oligomeric species. Co-treatment with the isotype control antibody B12 did not change this pattern of proteolysis or uptake.

5 **[000196]** In the media from cells treated with fibrils together with 37 there was mainly full length α -synuclein (14 kD) and only small amounts of the terminally truncated band (12 kD). In cell lysate from cells treated with fibrils together with 37 there was full length α -synuclein only, indicating that 37 prevent cleavage of FL- α -synuclein. Furthermore the total amount of FL- α -synuclein is reduced in
10 relation to cells treated with PFFs only or B12 control antibody. It has been shown by several groups (Games et al, Am J of Pathol, Vol. 182, No. 3, March, 2013; Ritchie et al, Health, Vol. 4, Special Issue, 1167-1177, 2012; Mishizen-Eberz, Biochemistry, 2005, 44, 7818-7829; Dufty et al, Am J of Pathol, Vol. 170, No. 5, May 2007) that α -synuclein can be cleaved by Calpain-1. The cleavage site of Calpain-1 for
15 fibrillized α -synuclein has been found to be in the region 114-122 (Mishizen-Eberz, J of Neurochem, 86, 836–847, 2003). In vivo in transgenic animals and human brain 1-119/122 seems to be the main cleavage product – in α -synuclein, the cleavage is likely after aspartate 119 or asparagine 122, which is deamidated to aspartate and is cleaved by Calpain or another protease with similar cleavage specificity. These results
20 indicate that antibody 37 is able to inhibit C-terminal truncation of α -synuclein. The epitope of antibody 37 overlaps with the enzyme Calpain-1 binding site, so binding of 37 to α -synuclein could directly inhibit binding and cleavage mediated by Calpain-1 (Fig 10 and 11).

[000197] The epitope of 285 overlaps with the epitope of 37 and it would also be
25 expected to inhibit the protease cleavage. The amino acid sequence of 37v2 only differs from 37 at one amino acid in CDR and has similar binding as 37, so it would also be expected to inhibit protease cleavage in similar manner to 37. To investigate if the effect of the antibodies were dose-dependent, a 24-hour experiment with co-addition of PFFs and antibodies to primary cortical neurons was set up. The
30 concentration of PFFs were stable (10 μ g/ml), whereas the concentrations of control antibody B12, and antibodies 37, 37v2 and 285 that was tested was 10, 5, 1 and 0,1 μ g/ml. α -synuclein on Western Blots were detected with 1904/4B12 antibody (Abcam), which has an epitope in the region 103-108 and therefore binds to both the

FL and the C-terminally truncated alpha-synuclein (Fig. 11B). As can be seen from Figure 11B, both GM37, 37v2 and GM285 have a dose dependent inhibition of protease cleavage, with almost complete inhibition of the cleavage at high concentration of the antibody.

5 **Example 6: Antibody-Mediated Inhibition Of Seeding Of Alpha-Synuclein Aggregation In Cell Culture**

[000198] Several studies have shown that exogenous addition of recombinant alpha synuclein fibrillar aggregates can enter cells and recruit endogenous alpha-synuclein and induce alpha-synuclein aggregation and phosphorylation in vitro and in vivo, which resemble LB. (Volpicelli-Daley et al. 2011, Luk et al. 2012a, Luk et al. 2012b, Recasens et al. 2013, Peelaerts et al. 2015). To study seeding of endogenous mouse alpha-synuclein by recombinant alpha-synuclein seeds, mouse primary cortical neurons prepared as above are plated in 96 well plates (15,000 cells per well). On day 5 in vitro culture (DIV), 50% of media is changed and supplemented with cytosine arabinoside (final conc. of 1uM). On DIV 6, half of the media is changed with glia conditioned media along with alpha synuclein fibrillary material, either crude fibril seeds or pure seeds. The crude fibril seeds are made from recombinant monomeric human alpha-synuclein, which was isolated from bacteria and the monomers were filtered through an Amicon Ultra 100.000 cut off filter (Millipore cat. No UFC510096) and adjusted to concentration of 1mg/ml in PBS, pH 7.4. To make fibril crude seeds, the monomer solution was incubated in thermomixer at 37C with continuous mixing (800rpm) until plateau is reached (evaluated by daily measures with Thioflavin S). To minimize evaporation a drop of mineral oil was added to cover the solution. The total time for incubation was 5-7 days, The pure seeds are made from crude fibril seeds that are centrifuged to purify them and the aggregated pellet is resuspended in fresh PBS and sonicated. The antibodies are added once on DIV 6 along with alpha-synuclein crude seeds. Half of the media in the primary neurons is replaced with glia conditioned media every week to maintain them up to DIV21. The neurons are fixed and stained for Phospho-synuclein using a rabbit antibody specific for phosphorylation of alpha-synuclein at amino acid S129 (abcam 51253), followed by a fluorescently labelled anti-rabbit antibody, fluorescence is quantified using automated fluorescent microscopy, Cellomics Arrayscan. Nuclei were detected in one channel and defined the number of valid cells. Phosphorylated alpha-synuclein spots were detected in another channel in

a pre-defined ring-formed area surrounding the nucleus, thus representing the cytoplasm of the cells. The average number of spots per cell was calculated. Example of cell staining is shown in Fig 12A. Phosphorylated alpha synuclein spots are not seen in untreated neurons. Neurons incubated with crude or pure seeds (1-10ng per well) induce phosphorylation of alpha synuclein (Fig. 12A). In neurites, phosphorylated synuclein appears as spots or punctate and some of the phospho-synuclein in the neurites appear elongated.

[000199] For fractionation studies cells were harvested in phosphate buffered saline solution (PBS) and centrifuged. Pellet was resuspended in 1% triton buffer with protease inhibitors. Samples were kept on ice for 15 min. followed by sonication. The samples were centrifuged at 100,000x g for 30 min. at 4C. The supernatant is collected and labelled as soluble fraction. The pellet was washed once in triton buffer and re-suspended in 1% SDS buffer followed by sonication. Samples were centrifuged again at 100,000xg for 30 min. The supernatant is collected as insoluble fraction. The protein concentrations were measured and samples were run on 4-12% SDS_PAGE gel, blotted on membranes and alpha synuclein and phosphorylated alpha synuclein (S129P) are detected by 4B12/1904 antibody (Thermo scientific: MA1-90346-human synuclein), S129P-asyn antibody (abcam 51253) and mouse synuclein antibody (cell signalling- D37A6), respectively.

[000200] Figure 12 B shows the Western blot of the soluble and insoluble fraction from the primary neurons with and without crude seeds. As can be seen from Figure 12B the addition of the seeds lead to accumulation of endogenous mouse alpha-synuclein and p-S129-alpha-synuclein and multimers of phosphorylated mouse alpha-synuclein in the insoluble fraction of the cells.

[000201] To test if antibodies can inhibit seeding, alpha synuclein synuclein seeds were used at conc. of 6.6nM (10ng/ well). Different concentration of antibody and alpha- synuclein seeds were added together on DIV 6, to make a dose response (starting from highest antibody conc. at 133nM down to 133 pM). The neurons were again fixed and stained for Phospho-synuclein (abcam 51253) and fluorescence from cells was quantified using automated fluorescent microscopy, Cellomics arrayscan. The spots/puncta per cell were counted in Cellomics arrayscan. As can be seen from Figure 12C, both antibody 37, 37v2 and antibody 285 reduced alpha synuclein

phosphorylation in neurons in a dose dependent manner with similar maximal inhibition for 37, 37v2 and 285 (around 70-75%) and EC50 around 5 nM. Fractionation of the cellular proteins to soluble and insoluble fraction after treatment with antibody at the highest concentration (133 nM) shows that both antibodies 37, 37v2 and 285 inhibited the truncation of the recombinant crude seeds and accumulation of C terminally truncated fragment (CT a-syn), and reduced the accumulation of phosphorylated endogenous mouse alpha-synuclein and aggregated forms of mouse alpha-synuclein in the insoluble fraction, as seen in Figure 12D.

10 **Example 7. Acute Electrophysiological Effects Of Alpha-Synuclein Antibodies in vivo**

[000202] High expression levels of human alpha-synuclein are present in the hippocampus of F28-sncα transgenic mice, a model overexpressing wildtype alpha-synuclein under the control of the mouse alpha-synuclein promotor (Westerlund M, et al. Mol Cell Neurosci. 2008 Dec; 39 (4):586-91). Assessment of synaptic transmission and plasticity in the CA1 area of the hippocampus in 4 to 6 months old male F28-sncα transgenic and age-matched control mice was performed by in vivo electrophysiology. The data shows that basal synaptic transmission is significantly impaired in F28-sncα transgenic compared to age-matched control mice (**Fig. 13**).

20 [000203] F28-sncα transgenic and age-matched control male mice (CRO breeding, Taconic Europe A/S) aged 4 to 6 months were single-housed in controlled temperature ($22 \pm 1.5^\circ\text{C}$) and humidity conditions (55-65%) and kept in a 12:12 hour light/dark cycle (lights on at 06:00h). Food and water were available ad libitum.

[000204] Animals were anesthetized with an intraperitoneal (i.p.) injection of urethane (1.2 g/kg). Mice were then mounted in a stereotaxic frame, their temperature adjusted to 37.5°C via a heating pad, and the skull was exposed. A platinum wire was placed in the frontal bone to act as a reference, and an additional hole was drilled for insertion of the recording and stimulating electrodes in the hippocampus, at the following coordinates according to the atlas of Paxinos and Franklin (Paxinos and Franklin's the Mouse Brain in Stereotaxic Coordinates, 4th Edition, 2001): recording, 1.5-1.7 mm posterior to Bregma, 1.0-1.2 mm lateral to the midline, 1.4-1.7 mm below the surface of the brain; stimulation, 1.8-2.0 mm posterior to Bregma, 1.5-1.7 mm

lateral to the midline, 1.5-1.7 mm below the surface of the brain. Animals were left in the stereotaxic frame through the whole duration of the recordings and their level of anesthesia was regularly checked.

[000205] Field potentials (fEPSP) were evoked in the CA1 by electrical stimulation of the Schaffer collateral every 30 s, and the depth of the recording electrode was adjusted until a negative fEPSP was recorded in response to a unipolar square pulse. The slope of the evoked fEPSP was measured between 30 and 70% of the maximum amplitude of the fEPSP.

[000206] Once an optimal fEPSP was induced, basal synaptic transmission was assessed by the relationship between stimulation intensity and slope of the evoked fEPSP (input-output relationship). The different intensities of stimulation were 0, 25, 50, 75, 100, 150, 200, 300, 400, and 500 μ A, and were applied successively in increasing order, with 2 to 3 repeats for each intensity. Basal synaptic transmission was found to be significantly impaired in F28-snca transgenic compared to age-matched control mice.

[000207] The identified impairments in basal synaptic transmission in F28-snca transgenic mice were used to test the GM37, GM285 and comparator h9E4 for their ability to block the alpha synuclein mediated effect.

[000208] Recordings were performed in all experiments 3 to 6 h following administration of a single dose of antibody at a dose of 15 mg/kg (i.p.). Basal synaptic transmission were recorded in both hippocampi in each animal when possible, and recorded as individual experiments.

[000209] Acute treatment with h9E4 induced a significant reversal of the impairment in basal synaptic transmission in F28-snca transgenic mice (Tg-snca + h9E4 vs. Tg-snca + PBS, $p=0.002$, **Fig. 14**). However, the reversal by h9E4 was only partial, as indicated by a significantly differentiation to basal synaptic transmission in littermates treated with PBS ($p=0.007$).

[000210] Acute treatment with GM37 induced a significant reversal of the impairment in basal synaptic transmission in F28-snca transgenic mice (Tg-snca + GM37 vs. Tg-snca + PBS, $p=0.004$, **Fig. 15**). Basal synaptic transmission in GM37-

treated transgenic mice was not significantly different from basal synaptic transmission in PBS-treated littermates, indicating a full reversal of the impairment (Fig 15).

[000211] GM285 also induced a significant reversal of the impairment in basal synaptic transmission in F28-snca transgenic mice (Fig 16). Basal synaptic transmission in GM285-treated transgenic mice was not significantly different from basal synaptic transmission in PBS-treated littermates, indicating a full reversal of the impairment.

Example 8. Microdialysis to assess human alpha-synuclein in the brain of awake freely moving animals

[000212] The push-pull microdialysis method was used to assess the levels of human alpha-synuclein in brain interstitial fluid (ISF). Mice were single-housed in controlled temperature ($22 \pm 1.5^\circ\text{C}$) and humidity conditions (55-65%) and kept on a 12:12 hour light/dark cycle (lights on at 06:00h). Food and water were available *ad libitum*. The current study was performed in the hippocampus of F28-snca transgenic mice (50-54 weeks old). To enable microdialysis in the hippocampus, mice were anaesthetized with isoflurane and an intracerebral guide cannula (CMA) was stereotactically implanted into the brain, positioning the microdialysis probe in the hippocampus (co-ordinates of probe tip: 3.1 mm posterior and 2.8 mm lateral from bregma, and 1.3 mm relative dura mater) according to the atlas of Paxinos and Franklin 2001. Anchor screws and acrylic cement were used for the fixation of the guide cannulas. After implantation of the cannula mice were allowed to recover from the surgery for 2-3 days before dialysis.

[000213] On the day of the experiment, a 2-mm, 1000 kDa cut-off CMA probe was inserted through the guide cannula. A probe was connected to a microdialysis peristaltic pump with two channels (MAB20; Microbiotech) and operated in push-pull mode. The inlet tubing of the microdialysis probe was connected to a peristaltic pump perfusing the probe with artificial cerebrospinal fluid (aCSF; in mM: 147 NaCl, 2.7 KCl, 1.2 CaCl_2 , 0.85 MgCl_2). The peristaltic pump was also connected to the outlet tubing in order to prevent perfusion fluid loss from the probe, by pulling the fluid through the tubing. As a perfusion buffer, 25% bovine albumin fraction V (Sigma) was diluted to 0.2 % with artificial CSF on the day of use and filtered through a 0.1- μm membrane. The actual flow rate of the pump was determined without having the probe connected. The

sample tubes were weighed before and after sampling for a given time period and the flow rate was calculated. The pump was then set to a constant flow of 1 $\mu\text{L}/\text{min}$. A 120 min sampling regimen was used throughout the experiment period. To avoid interference of tissue damage, the experimental window was set from 14 to 48 hr after probe implantation. 14-16 h after the start of the experiments, GM37, human 9E4 or isotype control(anti-HEL) were injected i.p. at 15 mg/kg, and an additional 6 samples (12h of collection) were collected. The dialysates were stored at -80 °C. Concentration of human alpha synuclein was determined by ELISA (Covance ELISA kit).

[000214] The average of the two-three basal values (4h-6h) prior to antibody treatment was taken as baseline and set to 100% for each animal. Data was evaluated using two-way analysis of variance (ANOVA) with repeated measures to evaluate statistical relevance. The basal levels of human alpha-synuclein in hippocampus were 8.1 ± 1.1 ng/ml (mean $\pm$ SEM, n = 25, not corrected for the in vitro dialysis probe recovery). The administration of GM37 induced a larger reduction in human alpha-synuclein in the hippocampus of F28 mice compared to both the comparator antibody, human 9E4, and the isotype control (anti-HEL). (**Fig. 17**).

Example 9: Chronic effects of alpha-synuclein antibodies in vivo. Antibody GM37 ameliorate motor phenotype in rat Parkinson model.

[000215] Targeted overexpression of human alpha-synuclein to dopaminergic neurons in the rat midbrain can be achieved using a recombinant adeno-associated viral vector (rAAV) and is associated with a progressive loss of dopaminergic cells in the substantia nigra as well as motor impairments.

[000216] Adult female Sprague-Dawley rats (225-250g) were used to express human alpha-synuclein in substantia nigra (SN) by injection with Adeno associated virus of AAV2/5 serotype containing chicken beta-actin promoter with enhancer elements from the cytomegalovirus promoter, followed by human alpha-synuclein cDNA and WPRE element as previously described (Xu L, Daly T, Gao C, Flotte TR, Song S, Byrne BJ, Sands MS, Ponder KP (2001). In this model it has been shown that human alpha-synuclein expression leads to neurodegeneration of dopaminergic neurons. Maingay M, et al. CNS Spectr. 2005 Mar;10(3):235-44). To test the effect of an alpha synuclein therapeutic antibody in this model antibody treatment was initiated 2 to 4 days prior to viral injections, and continued until the end of the study (**Fig. 18**).

PBS administration at the same volume (5 ml/kg; IP) was used as a control. GM37 was dosed twice per week at a dose of 15 mg/kg (IP). The viral particles (rAAV2/5) containing the gene for human wt alpha-synuclein or green fluorescent protein (GFP) were injected unilaterally in the SN. Animals were anaesthetized with a combination of Hypnorm® and Dormicum® at 2.0 ml/kg s.c. and placed in a stereotaxic frame. Their temperature was adjusted to 37.5 °C via a heating pad, and their skull was exposed. A hole was drilled above the right SN at the following coordinates, according to the atlas of Paxinos and Watson (Paxinos & Watson, 1998): 5.5 mm posterior and 2.0 mm lateral from Bregma. A single injection of 3 µL of rAAV2/5-alpha-syn or rAAV2/5-GFP was performed at a depth of 7.2 mm below the dura matter, and a flow rate of 0.2 µL/min using a Hamilton syringe connected to a stereotaxic injector. The needle was left in place an additional 5 min to allow diffusion of the vector in the SN. Following surgery, the animals were returned to their home cage, and placed in a heated environment where they were allowed to recover from anesthesia. Testing of motor asymmetry in the cylinder test was evaluated prior to AAV injections, as well as 3, 7 and 10 weeks following AAV injections. The data presented correspond to the ratio between use of the right forepaw compared to the total use of both the left and right forepaws. Each animal's performance in the cylinder was filmed for a total 5 min, and manual scoring of the number of touches using the left and right forepaws for 5 minutes has been performed for the final testing day 10 weeks after virus injection. A significant impairment is present in AAV-syn compared to AAV-GFP injected rats ($p=0.012$) at week 10. A trend for a reversal for GM37 treated animals was shown, as their performance is different from GFP rats ($p=0.163$ and $p=0.407$ for gm37, respectively). This finding indicates that antibody GM37 is able to ameliorate the Parkinsonian motor phenotype in this rat model (Figs 18 and 19).

Example 10: Chronic effects of alpha synuclein antibodies in vivo. Antibody GM37 inhibits seeding of endogenous mouse alpha synuclein aggregation and phosphorylation

[000217] Injection of alpha synuclein preformed fibrils made from recombinant protein into dorsal striatum of wild type mice recruit endogenous mouse alpha synuclein and induce formation of Ser-129 phosphorylated aggregates inside neurons in cortex, amygdala and substantia nigra (Luk et al. 2012, Science. 2012 Nov 16;338(6109):949-53). To see if alpha-synuclein specific monoclonal antibody GM37 could reduce the appearance of alpha-synuclein fibril-induced phosphorylated alpha

synuclein inclusion formation in vivo a total of 45 mice were used. Mice were dosed with GM 37 at 30 mg/kg i.p, GM 3715 mg/kg i.v. , or vehicle ip (PBS). One day later the mice were anesthetized and stereotactically injected in one hemisphere with 2 ul of recombinant human alpha-Syn crude seeds, made as described previously (Example 6) (total of 2 µg crude seeds per animal). To inject the crude seeds, the skull was opened by boring a hole and a single glass pipette was inserted (co-ordinates: +0.5 mm anterior to Bregma, +2.0 mm lateral to midline) into the right forebrain to target the inoculum to the dorsal neostriatum (+2.6 mm beneath the dura). Following recovery, the mice received weekly i.p. or i.v. injections of antibodies until sacrifice at 45 days.

Groups of 15 mice/group were dosed either iv w. GM37 15mg/kg, ip with GM37 30mg/kg, or PBS (10 ml/kg) ip once weekly.

[000218] To measure the antibody concentration in plasma, cheek blood was drawn once weekly just prior to next injection, ie 7 days after last injection. Plasma was obtained by a 2000g spin, 15min incubation at RT, supernatant was subsequently frozen at -20°C. A CSF sample was taken at the end of the study and frozen at -20 °C. Plasma and CSF samples were analysed to determine the concentration of Human IgG by MSD. In short, mouse anti-human IgG (clone MH16-1 (M1268) was used for capture, plasma or CSF was incubated in the well, followed by a sulfo-TAG goat anti-human as the detection antibody (MSD cat no: R32AJ-1). Plates were analysed from electrochemiluminescence by MSD.

[000219] The antibody levels in plasma are shown in Figure 20B and show a dose dependent increase in antibody plasma concentration and accumulation of antibody in plasma during the six weeks. The antibody levels in csf are shown in Figure 20C, and show that around 0.1% of antibody level in plasma can be measured in csf.

[000220] At day 45, from the time of the injection of the alpha synuclein fibrillary seed, the mice were anesthetized, transcardially perfused with PBS, followed by perfusion with neutral buffered paraformaldehyde (4%). The brains were removed and incubated overnight for post-fixation in neutral buffered paraformaldehyde. Immunohistochemistry was performed on 45 µm thick serial sections by Neuroscience associates. Briefly, Using MultiBrain® technology, up to 25 mouse brains were embedded together per block, into 3 blocks, freeze-sectioned at 45µm thickness in the coronal plane, and collected into cups containing antigen preserve solution. Every sixth

section was stained with antibody to Ser-129 phosphorylated alpha-synuclein (Anti-alpha Synuclein (phospho S129) antibody [Psyn/81A] ab184674) to reveal Ser129 phosphorylated alpha synuclein reactive structures.

- 5 **[000221]** Quantitation of pSyn pathology was performed by manual counting immunoreactive positive cells from images at 10 x magnification from 5-7 sections covering the entire substantia nigra from every sixth section. The counting was performed blinded. Cell counts in amygdala and nigra were analysed by a one-way ANOVA followed by Bonferoni t-test, where the effect of GM37 antibody was compared to PBS treatment.
- 10 **[000222]** As can be seen from Fig 20C, treatment with antibody GM37 reduced the number of intra-cellular inclusions in Substantia Nigra significantly when compared to PBS control, with either ip or iv treatment. The data shows that antibody GM37 could have therapeutic effect in PD by blocking entry of extracellular pathological alpha-synuclein into neurons, by blocking its propagation between neurons and/or facilitating
- 15 clearance from the ISF by uptake into microglia. As this appearance of inclusions has been linked to loss of dopaminergic neurons and development of Parkinsonian motor deficits in animal models, treatment with antibody GM37 could have a therapeutic effect on loss of dopaminergic cells and development of motor deficits in PD.

Example 11: Manufactability of GM37 and GM37 variants

- 20 **[000223]** The anti-alpha-synuclein antibodies are 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
- 25 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
- 30 sequences and determine the potential risk they will have on the manufacturability and developability of a therapeutic antibody.

[000224] 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 identify unique amino acids that are deemed important for the molecule's 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.

[000225] We identified a site for deamidation within the VH domain of GM37. Amino acid residue 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 methods (example 1.5). All 3 variants showed similar expression and purification properties as GM37wt. (**Fig. 23**).

[000226] 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 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, 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, 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).

[000227] 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 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. 24**). 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®).

[000228] 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. 25**).

[000229] In order to evaluate the impact of the changes at N54 on the functional activity of GM37 we analyzed the ability of the antibodies to block synuclein seeding activity in a culture of primary neurons (**Example 6**). The level of seeding is measured using an antibody specific for phospho-synuclein . All 3 antibodies were able to block seeding as measured by the phospho-synuclein signal (**Fig. 26**). Furthermore, the level of inhibition was the same for all 4 antibodies. This call based

data further confirms the binding data that amino acid 54 in the VH domain is not required for binding affinity to human alpha-synuclein or for inhibition of seeding in a primary cell based assay. Furthermore, we found that all three of these antibodies were capable of production using standard expression and purification methods.

- 5 Interestingly one of the variants N54Q showed improvement in production over the other variants, which is of great importance when the antibody is to be produced commercially on large scale. These data support the possibility of reducing the potential risk of deamidation by replacing asparagine (N) with another amino acid without concern over the loss of potency.

- 10 **[000230]** A samples of each of the antibodies wt GM37, var1, var2 and var3 was subjected to a steady increase in temperature over time and the level of aggregation was simultaneously measured by multi-angle light scattering (Prometheus NT.48, NanoTemper Technologies). The temperature for onset of aggregation was found to be similar for GM37 and the GM37-variants, however the lowest level of aggregation
15 observed for GM37-Var2 (**Fig. 27**).

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 35 <400> 2
 40 Ala Ile Arg Ser Asn Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
 1 5 10 15
 Gly
 45 <210> 3
 <211> 9
 <212> PRT
 <213> Artificial
 50 <220>
 <223> GM37 CDR3 Heavy Chain
 <400> 3
 55 Ala Lys Asn Trp Ala Pro Phe Asp Ser
 1 5
 60 <210> 4
 <211> 11
 <212> PRT
 <213> Artificial

<220>
 <223> GM37 CDR1 Light Chain
 5 <400> 4
 Ala Ser Gln Ser Val Ser Ser Ser Tyr Leu Ala
 1 5 10
 10
 <210> 5
 <211> 7
 <212> PRT
 <213> Artificial
 15
 <220>
 <223> GM37 CDR 2 Light Chain
 <400> 5
 20 Gly Ala Ser Ser Arg Ala Thr
 1 5
 25 <210> 6
 <211> 9
 <212> PRT
 <213> Artificial
 30 <220>
 <223> GM37 CDR 3 Light Chain
 <400> 6
 35 Gln Gln Tyr Gly Ser Ser Pro Trp Thr
 1 5
 40 <210> 7
 <211> 116
 <212> PRT
 <213> Artificial
 45 <220>
 <223> GM37 CDR Heavy Chain
 <400> 7
 50 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
 1 5 10 15
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 55 Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 60 Ser Ala Ile Arg Ser Asn 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
 65 70 75 80

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

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

Thr Val Ser Ser
 115

15 <210> 8
 <211> 108
 <212> PRT
 20 <213> Artificial

<220>
 <223> GM 37 Light Chain

25 <400> 8

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

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

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

40 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
 65 70 75 80

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

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

55 <210> 9
 <211> 6
 <212> PRT
 <213> Artificial

60 <220>
 <223> Epitope 112-117
 <400> 9

Ile Leu Glu Asp Met Pro
 1 5

5 <210> 10
 <211> 140
 <212> PRT
 <213> Artificial

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

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

20 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

25 val His Gly val Ala 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 Ser Ile Ala Ala Ala Thr Gly Phe val Lys
 85 90 95

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

45 Leu Glu Asp Met Pro val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125

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

50 <210> 11
 <211> 165
 <212> PRT
 <213> Artificial

55 <220>
 <223> A-Syn-AAKK-BAP
 <400> 11

60 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 25 30

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

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

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

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

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

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

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

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

Ile Glu Trp His Glu
165

40 <210> 12
<211> 165
<212> PRT
<213> Artificial

45 <220>
<223> A-Syn-BAAK-BAP

50 <400> 12

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

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

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

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

5 Asn val Gly Gly Ala val val Thr Gly val Thr Ala val Ala Gln Lys
 65 70 75 80
 Thr val Glu Gly Ala Gly Ser Ile 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
 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
 Ile Glu Trp His Glu
 165
 30 <210> 13
 <211> 162
 <212> PRT
 <213> Artificial
 35 <220>
 <223> A-Syn-BBAA-BAP
 <400> 13
 40 Met Asp val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly val val
 1 5 10 15
 45 Ala Ala Ala Glu Lys Thr Lys Gln Gly val Thr Glu Ala Ala Glu Lys
 20 25 30
 50 Thr Lys Glu Gly val Leu Tyr val Gly Ser Lys Thr Arg Glu Gly val
 35 40 45
 val Gln Gly val Ala Ser val Ala Glu Lys Thr Lys Glu Gln Ala Ser
 50 55 60
 55 His Leu Gly Gly Ala val val Thr Gly val Thr Ala val Ala Gln Lys
 65 70 75 80
 60 Thr val Glu Gly Ala Gly Ser Ile Ala Ala Thr Gly Phe val Lys
 85 90 95

Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110
 5 Leu Glu Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125
 10 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala Gly Ser Ala Gly
 130 135 140
 15 Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp
 145 150 155 160
 His Glu
 20
 <210> 14
 <211> 165
 <212> PRT
 <213> Artificial
 25
 <220>
 <223> A-Syn-BBKK-BAP
 <400> 14
 30 Met Asp Val Phe Met Lys Gly Leu Ser Met Ala Lys Glu Gly Val Val
 1 5 10 15
 35 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Thr Glu Ala Ala Glu Lys
 20 25 30
 40 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Arg Glu Gly Val
 35 40 45
 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
 65 70 75 80
 50 Thr Val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Leu Val Lys
 85 90 95
 55 Lys Asp Gln Leu Ala Lys Gln Asn Glu Glu Gly Phe Leu Gln Glu Gly
 100 105 110
 60 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

5 Ser Ala Gly Gly Ser Gly Gly Leu Asn Asp Ile Phe Glu Ala Gln Lys
 145 150 155 160
 Ile Glu Trp His Glu
 165
 10 <210> 15
 <211> 141
 <212> PRT
 <213> Artificial
 15 <220>
 <223> A-Syn-120-140_De1-BAP
 <400> 15
 20 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15
 25 Ala Ala Ala Glu Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys
 20 25 30
 30 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 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
 40 Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys
 85 90 95
 45 Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110
 50 Leu Glu Asp Met Pro Val Asp Gly Ser Ala Gly Gly Ser Gly Gly Leu
 115 120 125
 55 Asn Asp Ile Phe Glu Ala Gln Lys Ile Glu Trp His Glu
 130 135 140
 60 <210> 16
 <211> 131
 <212> PRT
 <213> Artificial
 <220>
 <223> alpha-synuclein amino acids 1-119

<400> 16
 Met Ala His His His His His Ile Glu Gly Arg Met Asp Val Phe
 1 5 10 15
 5 Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val Ala Ala Glu
 20 25 30
 10 Lys Thr Lys Gln Gly Val Ala Glu Ala Ala Gly Lys Thr Lys Glu Gly
 35 40 45
 15 Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val Val His Gly Val
 50 55 60
 20 Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr Asn Val Gly Gly
 65 70 75 80
 Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys Thr Val Glu Gly
 85 90 95
 25 Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Val Lys Lys Asp Gln Leu
 100 105 110
 30 Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile Leu Glu Asp Met
 115 120 125
 35 Pro Val Asp
 130
 40 <210> 17
 <211> 106
 <212> PRT
 <213> artificial
 <220>
 45 <223> kappa (LC constant region)
 <400> 17
 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1 5 10 15
 50 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 20 25 30
 55 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 35 40 45
 60 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
 <210> 18
 <211> 329
 15 <212> PRT
 <213> Artificial
 <220>
 20 <223> IgG1 (HC Constant region)
 <400> 18
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15
 25 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 30 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 35 40 45
 35 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 40 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 45 Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 100 105 110
 50 Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 115 120 125
 55 Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 130 135 140
 60 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
 165 170 175

[illegible]

<400> 20
 Ala Ala Ser Gly Phe Thr Phe Ser Arg Phe Thr Met Thr
 1 5 10
 5
 <210> 21
 <211> 17
 <212> PRT
 10 <213> Artificial
 <220>
 <223> GM285 CDR2 Heavy Chain
 15 <400> 21
 Ala Ile Ser Gly Ser Gly Gly Gly Thr Ser Tyr Ala Asp Ser Val Lys
 1 5 10 15
 20 Gly
 25 <210> 22
 <211> 9
 <212> PRT
 <213> Artificial
 30 <220>
 <223> GM285 CDR3 Heavy Chain
 <400> 22
 35 Ala Lys Asn Trp Ala Pro Phe Asp Tyr
 1 5
 40 <210> 23
 <211> 12
 <212> PRT
 <213> Artificial
 <220>
 45 <223> GM285 CDR1 Light Chain
 <400> 23
 50 Arg Ala Ser Gln Ser Val Ser Arg Ser Tyr Leu Ala
 1 5 10
 55 <210> 24
 <211> 7
 <212> PRT
 <213> Artificial
 <220>
 60 <223> GM285 CDR2 Light Chain
 <400> 24
 Gly Ala Ser Ser Arg Ala Thr
 1 5

5 <210> 25
 <211> 9
 <212> PRT
 <213> Artificial

 10 <220>
 <223> GM285 CDR3 Light Chain
 <400> 25
 Gln Gln Tyr Gly Ser Ser Pro Trp Thr
 1 5

 20 <210> 26
 <211> 116
 <212> PRT
 <213> Artificial

 25 <220>
 <223> GM285 VH
 <400> 26
 Glu Val Gln Leu Leu 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 Arg Phe
 20 25 30
 35 Thr Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 40 Ser Ala Ile Ser Gly Ser Gly Gly Gly Thr Ser Tyr Ala Asp Ser Val
 50 55 60
 45 Lys Gly Arg Leu Thr Val Ser Arg Asp Asn Ser Lys 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
 50 Ala Lys Asn Trp Ala Pro Phe Asp Tyr Trp Gly Gln Gly Thr Leu Val
 100 105 110
 55 Thr Val Ser Ser
 115
 60 <210> 27
 <211> 108
 <212> PRT
 <213> Artificial
 <220>

<223> GM285 VL
 <400> 27
 5 Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly
 1 5 10 15
 10 Glu Arg Ala Thr Leu Ser Cys Arg Ala Ser Gln Ser Val Ser Arg Ser
 20 25 30
 15 Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg Leu Leu
 35 40 45
 Ile Tyr Gly Ala Ser Ser Arg Ala Thr Gly Ile Pro Asp Arg Phe Ser
 50 55 60
 20 Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Val Ser Arg Leu Glu
 65 70 75 80
 25 Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Tyr Gly Ser Ser Pro
 85 90 95
 30 Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
 100 105
 <210> 28
 <211> 329
 35 <212> PRT
 <213> Artificial
 <220>
 <223> GM285 IgG1 constant region
 40 <400> 28
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15
 45 Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 50 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 55 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 60 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

5 Arg Val Glu Pro₁₀₀ Lys Ser Cys Asp Lys₁₀₅ Thr His Thr Cys Pro₁₁₀ Pro Cys
 Pro Ala Pro₁₁₅ Glu Leu Leu Gly Gly₁₂₀ Pro Ser Val Phe Leu₁₂₅ Phe Pro Pro
 10 Lys Pro₁₃₀ Lys Asp Thr Leu Met₁₃₅ Ile Ser Arg Thr Pro₁₄₀ Glu Val Thr Cys
 15 Val₁₄₅ Val Val Asp Val Ser₁₅₀ His Glu Asp Pro Glu₁₅₅ Val Lys Phe Asn Trp₁₆₀
 20 Tyr Val Asp Gly Val₁₆₅ Glu Val His Asn Ala₁₇₀ Lys Thr Lys Pro Arg Glu₁₇₅
 Glu Gln Tyr Asn₁₈₀ Ser Thr Tyr Arg Val₁₈₅ Val Ser Val Leu Thr₁₉₀ Val Leu
 25 His Gln Asp Trp Leu Asn Gly Lys₂₀₀ Glu Tyr Lys Cys Lys₂₀₅ Val Ser Asn
 30 Lys Ala₂₁₀ Leu Pro Ala Pro Ile₂₁₅ Glu Lys Thr Ile Ser₂₂₀ Lys Ala Lys Gly
 35 Gln Pro Arg Glu Pro Gln₂₃₀ Val Tyr Thr Leu Pro₂₃₅ Pro Ser Arg Glu Glu₂₄₀
 40 Met Thr Lys Asn Gln₂₄₅ Val Ser Leu Thr Cys₂₅₀ Leu Val Lys Gly Phe Tyr₂₅₅
 Pro Ser Asp Ile₂₆₀ Ala Val Glu Trp Glu₂₆₅ Ser Asn Gly Gln Pro₂₇₀ Glu Asn
 45 Asn Tyr Lys₂₇₅ Thr Thr Pro Pro Val₂₈₀ Leu Asp Ser Asp Gly₂₈₅ Ser Phe Phe
 50 Leu Tyr₂₉₀ Ser Lys Leu Thr Val₂₉₅ Asp Lys Ser Arg Trp₃₀₀ Gln Gln Gly Asn
 55 Val₃₀₅ Phe Ser Cys Ser Val₃₁₀ Met His Glu Ala Leu₃₁₅ His Asn His Tyr Thr₃₂₀
 60 Gln Lys Ser Leu Ser₃₂₅ Leu Ser Pro Gly
 <210> 29
 <211> 106

<212> PRT
 <213> Artificial
 5 <220>
 <223> GM285 kappa chain
 <400> 29
 10 Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
 1 5 10 15
 Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
 20 25 30
 Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
 35 40 45
 20 Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 50 55 60
 25 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
 65 70 75 80
 30 His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
 85 90 95
 Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 100 105
 35
 <210> 30
 <211> 116
 <212> PRT
 40 <213> Artificial
 <220>
 <223> GM37 variant 1 heavy chain
 45 <400> 30
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
 1 5 10 15
 50 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
 20 25 30
 55 Ala Met Thr Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 60 Ser Ala Ile Arg Ser Ser 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
 65 70 75 80

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 Ser Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr val Ser Ser
115

<210> 31
<211> 116
<212> PRT
<213> Artificial

<220>
<223> GM 37 variant 2 heavy chain

<400> 31

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

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
35 40 45

Ser Ala Ile Arg Ser Gln 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
65 70 75 80

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 Ser Trp Gly Gln Gly Thr Leu Val
100 105 110

Thr val Ser Ser
115

<210> 32
<211> 116
<212> PRT
<213> Artificial

<220>
<223> GM 37 variant 3 heavy chain

<400> 32
 Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Thr Gly Gly
 1 5 10
 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
 35 40 45
 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
 65 70 75 80
 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 Ser Trp Gly Gln Gly Thr Leu Val
 100 105 110
 Thr Val Ser Ser
 115
 <210> 33
 <211> 17
 <212> PRT
 <213> Artificial
 <220>
 <223> GM37 variant 1 heavy chain CDR 2
 <400> 33
 Ala Ile Arg Ser Ser Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
 1 5 10 15
 Gly
 50
 <210> 34
 <211> 17
 <212> PRT
 <213> Artificial
 <220>
 <223> GM37 variant 2 CDR 2 heavy chain
 60
 <400> 34
 Ala Ile Arg Ser Gln Gly Asp Arg Thr Asp Tyr Ala Asp Ser Val Lys
 1 5 10 15

Gly

5

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

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

15 <400> 35

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

20 Gly

25 <210> 36
 <211> 5
 <212> PRT
 <213> Artificial

30 <220>
 <223> 9E4 binding epitope

<400> 36

35 Asn Glu Ala Tyr Glu
 1 5

40 <210> 37
 <211> 134
 <212> PRT
 <213> Artificial

<220>
 45 <223> HUMAN Beta-synuclein

<400> 37

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 25 30

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

60 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 Phe Ser Gly Ala Gly Asn Ile Ala Ala Ala
 65 70 75 80

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

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

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

Glu Tyr Glu Pro Glu Ala
 130

20 <210> 38
 <211> 127
 <212> PRT
 <213> Artificial

25 <220>
 <223> HUMAN Gamma-synuclein

30 <400> 38
 Met Asp Val Phe Lys Lys Gly Phe Ser Ile Ala Lys Glu Gly Val Val
 1 5 10 15

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

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

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

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

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

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

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

<210> 39
 <211> 140

<212> PRT
 <213> Artificial

5 <220>
 <223> alpha-synuclein ortholog for Cynomolgus monkey
 <400> 39

10 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
 15 20 25 30
 Thr Lys Glu Gly Val Leu Tyr Val Gly Ser Lys Thr Lys Glu Gly Val
 35 40 45
 20 Val His Gly Val Ala Thr Val Ala Glu Lys Thr Lys Glu Gln Val Thr
 50 55 60
 25 Asn Val Gly Gly Ala Val Val Thr Gly Val Thr Ala Val Ala Gln Lys
 65 70 75 80
 30 Thr Val Glu Gly Ala Gly Ser Ile Ala Ala Ala Thr Gly Phe Ile Lys
 85 90 95
 Lys Asp Gln Leu Gly Lys Asn Glu Glu Gly Ala Pro Gln Glu Gly Ile
 100 105 110
 35 Leu Gln Asp Met Pro Val Asp Pro Asp Asn Glu Ala Tyr Glu Met Pro
 115 120 125
 40 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140

45 <210> 40
 <211> 140
 <212> PRT
 <213> Artificial

50 <220>
 <223> alpha-synuclein ortholog for Rat
 <400> 40

55 Met Asp Val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly Val Val
 1 5 10 15
 60 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 Thr 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
 65 70 75 80
 10 Thr val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe val Lys
 85 90 95
 15 Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110
 20 Leu Glu Asp Met Pro val Asp Pro Ser Ser 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
 <210> 41
 <211> 140
 <212> PRT
 30 <213> Artificial
 <220>
 <223> alpha-synuclein ortholog for Mouse
 35 <400> 41
 Met Asp val Phe Met Lys Gly Leu Ser Lys Ala Lys Glu Gly val val
 1 5 10 15
 40 Ala Ala Ala Glu Lys Thr Lys Gln Gly val Ala Glu Ala Ala Gly Lys
 20 25 30
 45 Thr Lys Glu Gly val Leu Tyr val Gly Ser Lys Thr Lys Glu Gly val
 35 40 45
 50 val His Gly val Thr 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
 65 70 75 80
 55 Thr val Glu Gly Ala Gly Asn Ile Ala Ala Ala Thr Gly Phe val Lys
 85 90 95
 60 Lys Asp Gln Met Gly Lys Gly Glu Glu Gly Tyr Pro Gln Glu Gly Ile
 100 105 110

Leu Glu Asp Met Pro Val Asp Pro Gly Ser Glu Ala Tyr Glu Met Pro
 115 120 125
 5 Ser Glu Glu Gly Tyr Gln Asp Tyr Glu Pro Glu Ala
 130 135 140
 10 <210> 42
 <211> 446
 <212> PRT
 <213> artificial
 15 <220>
 <223> 9E4 HC
 <400> 42
 20 Glu Val Gln Leu Val 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 Asn Tyr
 20 25 30
 Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
 35 40 45
 30 Ala Ser Ile Ser Ser Gly Gly Gly Ser Thr Tyr Tyr Pro Asp Asn Val
 50 55 60
 35 Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
 65 70 75 80
 40 Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys
 85 90 95
 45 Ala Arg Gly Gly Ala Gly Ile Asp Tyr Trp Gly Gln Gly Thr Leu Val
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 Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala
 115 120 125
 50 Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu
 130 135 140
 55 Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly
 145 150 155 160
 60 Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser
 165 170 175
 Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu
 180 185 190

5 Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr
 195 200 205
 10 Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
 210 215 220
 15 Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe
 225 230 235 240
 20 Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro
 245 250 255
 25 Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp Pro Glu Val
 260 265 270
 30 Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr
 275 280 285
 35 Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val
 290 295 300
 40 Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys
 305 310 315 320
 45 Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser
 325 330 335
 50 Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro
 340 345 350
 55 Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val
 355 360 365
 60 Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly
 370 375 380
 65 Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp
 385 390 395 400
 70 Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp
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 80 Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
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 10 <220>
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 20 Asp Arg Val Thr Ile Thr Cys Lys Ser Ile Gln Thr Leu Leu Tyr Ser
 20 20 25 30
 25 Ser Asn Gln Lys Asn Tyr Leu Ala Trp Phe Gln Gln Lys Pro Gly Lys
 35 40 45
 30 Ala Pro Lys Leu Leu Ile Tyr Trp Ala Ser Ile Arg Lys Ser Gly Val
 50 55 60
 35 Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
 65 70 75 80
 40 Ile Ser Ser Leu Gln Pro Glu Asp Leu Ala Thr Tyr Tyr Cys Gln Gln
 85 90 95
 45 Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
 100 105 110
 50 Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp
 115 120 125
 55 Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn
 130 135 140
 60 Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu
 145 150 155 160
 65 Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp
 165 170 175
 70 Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr
 180 185 190
 75 Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 195 200 205

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

CLAIMS

1. Use of a monoclonal antibody capable of specifically binding to human alpha-synuclein, 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
5 fragment thereof that binds said epitope, wherein the antibody, or antigen-binding fragment thereof is selected from the group consisting of antibody (1)-(15):

Antibody (1) or an antigen-binding 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: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
(f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

- 15 Antibody (2) comprises the heavy chain variable domain of **SEQ ID NO:7** or the light chain variable domain of **SEQ ID NO:8**;

Antibody (3) comprises 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**;

20

Antibody (4) or an antigen-binding 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**;
(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
(f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:6**;

- 30 Antibody (5) comprises the heavy chain variable domain of **SEQ ID NO:30** or the light chain variable domain of **SEQ ID NO:8**;

Antibody (6) comprises a heavy chain consisting of a variable domain of **SEQ ID NO:30** and a light chain consisting of a variable domain of **SEQ ID NO:8**;

Antibody (7) or an antigen-binding 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:34**;
- (c) a Heavy Chain CDR3 having the amino acid sequence of **SEQ ID NO:3**;
- 5 (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**;

10 Antibody (8) comprises the heavy chain variable domain of **SEQ ID NO:31** or the light chain variable domain of **SEQ ID NO:8**;

Antibody (9) comprises a heavy chain consisting of a variable domain of **SEQ ID NO:31** and a variable domain of **SEQ ID NO:8**;

15 Antibody (10) or an antigen-binding 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:35**;
- (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**;
- 20 (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 (11) comprises the heavy chain variable domain of **SEQ ID NO:32** or the light chain variable domain of **SEQ ID NO:8**;

25

Antibody (12) comprises a heavy chain consisting of a variable domain of **SEQ ID NO:32** and a variable domain of **SEQ ID NO:8**;

Antibody (13) or an antigen-binding fragment thereof, comprises:

- 30 (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**;
- (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

(f) a Light Chain CDR3 having the amino acid sequence of **SEQ ID NO:25**;

5 Antibody (14) comprises a heavy chain variable domain of **SEQ ID NO:26** or the light chain variable domain of **SEQ ID NO:27**;

Antibody (15) comprises a heavy chain consisting of a variable domain of **SEQ ID NO:26** and a variable domain of **SEQ ID NO:27**;

10 in the manufacture of a medicament for treatment together with an additional medicament selected from the group comprising dopamine, antibodies against any form of A β peptides, LAG-3 antibodies, Isradipine, nicotine, caffeine, Levodopa/carbidopa, Prlnacasan, Alicapistat, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, epigallocatechin gallate, Bapineuzemab,
15 Solanezumab, Gantenerumab, Crenezumab, Aducanumab, Relatlimab, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat , Rasagiline, Eldepryl, Zelapar, , Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline, wherein said treatment is of Parkinson's disease (including idiopathic and inherited forms of Parkinson's disease), Gauchers Disease (GD),
20 Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson's disease, pure autonomic failure and multiple system atrophy in a subject in need thereof.

2. The use according to claim 1, wherein said additional medicament is selected from the group comprising pramipexole, Prlnacasan, Alicapistat, PF-06447475,
25 NB-360, Neuropore NPT100-18A, NPT200-11, epigallocatechin gallate, Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, Relatlimab, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat, Rasagiline, Eldepryl, Zelapar, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline, or a pharmaceutically acceptable salt thereof.

3. The use according to any one of the preceding claims, wherein the antibody is a human, humanized, recombinant or chimeric antibody.
4. The use according to claim 1, wherein the treatment is chronic.

5. The use according to claim 4, wherein the chronic treatment is for at least 2 weeks.
6. The use according to claim 1, wherein the subject is human.
- 5 7. The use according to claims 1-6 wherein said monoclonal antibody is selected from antibody (1)-(15) as defined in claim 1, and additional medicament are to be administered approximately simultaneously or wherein the medicaments are to be taken separately.
- 10 8. A kit comprising any of antibodies (1) – (15) as defined in claim 1 and a medicament selected from the group comprising dopamine, antibodies against any form of A β peptides, LAG-3 antibodies, Isradipine, nicotine, caffeine, Levodopa/carbidopa, Pralnacasan, Alicapistat, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, epigallocatechin gallate, Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, , Relatlimab, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat ,
15 Rasagiline, Eldepryl, Zelapar, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefyline.
- 20 9. The kit according to claim 8 wherein said medicament is selected from the group comprising pramipexole, Pralnacasan, Alicapistat, PF-06447475, NB-360, Neuropore NPT100-18A, NPT200-11, epigallocatechin gallate, Bapineuzemab, Solanezumab, Gantenerumab, Crenezumab, Aducanumab, Relatlimab, GSK2831781, IMP321, ELN484228, NCGC607, Miglustat, Eliglustat, Rasagiline, Eldepryl, Zelapar, Ropinerole, Bromocriptine, Comtess, Tasmar and Istradefylin, or a pharmaceutically acceptable salt thereof.