(19) DANMARK

(10) DK/EP 2807188 T3

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: *C 07 K 16/18 (2006.01)* *A 61 K 39/00 (2006.01)* *A 61 K 49/00 (2006.01)* *A 61 K 49/16 (2006.01)* *A 61 K 51/10 (2006.01)*

(45) Oversættelsen bekendtgjort den: **2019-10-07**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2019-09-11**

(86) Europæisk ansøgning nr.: **13740871.2**

(86) Europæisk indleveringsdag: **2013-01-25**

(87) Den europæiske ansøgnings publiceringsdag: **2014-12-03**

(86) International ansøgning nr.: **US2013023307**

(87) Internationalt publikationsnr.: **WO2013112945**

(30) Prioritet: **2012-01-27 US 201261591835 P** **2012-10-08 US 201261711207 P**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

(73) Patenthaver: **Prothena Biosciences Limited, 77 Sir Rogerson's Quay , Block C , Grand Canal Docklands, Dublin 2, D02 T804, Irland**

(72) Opfinder: **SALDANHA, Jose, 21 Fillebrook Ave., Enfield EN1 3BD, Storbritannien**
NIJJAR, Tarlochan S., 34 Owl Hill Rd., Orinda, California 94563, USA

(74) Fuldmægtig i Danmark: **NORDIC PATENT SERVICE A/S, Bredgade 30, 1260 København K, Danmark**

(54) Benævnelse: **HUMANISEREDE ANTISTOFFER, DER GENKENDER ALPHA-SYNUCLEIN**

(56) Fremdragne publikationer:
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WO-A2-2004/039234
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Fortsættes ...

Description

BACKGROUND

[0001] Synucleinopathies, including Lewy body diseases (LBDs) are characterized by degeneration of the dopaminergic system, motor alterations, cognitive impairment, and formation of Lewy bodies (LBs) and/or Lewy neurites. (McKeith et al., Neurology (1996) 47:1113-24). Synucleinopathies include Parkinson's disease (including idiopathic Parkinson's disease), Diffuse Lewy Body Disease (DLBD) also known as Dementia with Lewy Bodies (DLB), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson disease, pure autonomic failure and multiple system atrophy (MSA; e.g., Olivopontocerebellar Atrophy, Striatonigral Degeneration and Shy-Drager Syndrome). Several non-motor signs and symptoms are thought to be harbingers for synucleinopathies in the prodromal phase of the diseases (i.e., the presymptomatic, subclinical, preclinical, or premotor period). Such early signs include, for example, REM sleep behavior disorder (RBD), loss of smell and constipation (Mahowald et al., Neurology (2010) 75:488-489). Lewy body diseases 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).

[0002] Alpha-synuclein is part of a large 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 neural plasticity, learning and memory. Several studies have implicated alpha-synuclein with a central role in PD pathogenesis. The protein can aggregate to form 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 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). Over expression of alpha synuclein in transgenic mice (Masliah et al., Science (2000) 287:1265-9) and Drosophila (Feany et al., Nature (2000) 404:394-8) mimics several pathological aspects of Lewy body disease. In addition, it has been suggested that soluble oligomers of synuclein may be neurotoxic (Conway et al., Proc. Natl. Acad. Sci. USA (2000) 97:571-576; Volles et al., J. Biochemistry (2003) 42:7871-7878). The accumulation of alpha-synuclein with similar morphological and neurological alterations in species and animal models as diverse as humans, mice, and flies suggests that this molecule contributes to the development of Lewy body disease. US20090208487-A discusses agents and methods for treatment of diseases associated with synucleinopathic diseases, including Lewy bodies of alpha-synuclein in the brain of a patient. Several murine anti-synuclein monoclonal antibodies are discussed.

SUMMARY OF THE CLAIMED INVENTION

[0003] The invention provides an antibody comprising a mature heavy chain variable region having an amino acid sequence designated SEQ ID NO:23 and a light chain variable region having an amino acid sequence designated SEQ ID NO:33 or 37. In some of such antibodies, the K_D for alpha-synuclein of the antibody is from about 0.5 to 2 of K_D for alpha-synuclein of a murine or chimeric 1H7 antibody. In some of such antibodies, the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:23 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO:37.

[0004] In some antibodies, the mature heavy chain variable region has an amino acid sequence designated SEQ ID NO:23 and the mature light chain variable region has an amino acid sequence designated SEQ ID NO: 33.

[0005] In any of the above antibodies, the mature heavy chain variable region can be fused to a heavy chain constant region and the mature light chain constant region can be fused to a light chain constant region.

[0006] In any of the above antibodies, the heavy chain constant region can be a mutant form of natural human constant region which has reduced binding to an Fcγ receptor relative to the natural human constant region.

[0007] In any of the above antibodies, the heavy chain constant region can be of human IgG1 isotype. In some antibodies the allotype is G1m3. In some antibodies, the allotype is G1m1.

[0008] In some antibodies, the heavy chain constant region has the amino acid sequence designated SEQ ID NO:52 provided the C-terminal lysine residue may be omitted. In some antibodies, the light chain constant region has the amino acid sequence designated SEQ ID NO:49. In some antibodies, the mature heavy chain variable region is fused to a heavy chain constant region having the amino acid sequence designated SEQ ID NO:52 provided the C-terminal lysine residue may be omitted and the mature light chain constant region is fused to a light chain constant region having the amino acid sequence designated SEQ ID NO:49. In some antibodies, the mature light chain comprises SEQ ID NO:53 and the mature heavy chain comprises SEQ ID NO:56.

[0009] The invention further provides a nucleic acid encoding any of the above-mentioned mature heavy chain variable regions and/or any of the above-mentioned mature light chain variable region, e.g., SEQ ID NOS: 22, 32 or 36.

[0010] The invention further provides a host cell comprising a vector comprising any of the nucleic acids described above.

[0011] The invention further provides a pharmaceutical composition comprising any of the above-mentioned antibodies.

[0012] The invention further provides the antibody of the invention for use in a method of treating a patient having or at risk of a Lewy body disease, the method comprising administering to the patient an effective regime of any of the above-mentioned antibodies. In some methods, the disease is Parkinson's disease. In some methods, decline of cognitive function in the patient is inhibited. In some methods, neuritic and/or axonal alpha synuclein aggregates are reduced. In some methods, neuritic dystrophy in the patient is reduced. In some methods, synaptic and/or dendritic density is preserved. In some methods, the method preserves synaptophysin and/or MAP2 in the patient.

[0013] The disclosure further provides a method of reducing Lewy body formation in a patient having or at risk of a Lewy body disease, comprising administering to the patient an effective amount of any of the above-mentioned antibodies. In some methods, the disease is Parkinson's disease. In some methods, decline of cognitive function in the patient is inhibited. In some methods, neuritic and/or axonal alpha synuclein aggregates are reduced. In some methods, neuritic dystrophy in the patient is reduced. In some methods, synaptic and/or dendritic density is preserved. In some methods, the method preserves synaptophysin and/or MAP2 in the patient.

[0014] The disclosure further provides a method of inhibiting synuclein aggregation or clearing Lewy bodies or synuclein aggregates in a patient having or at risk of a Lewy body disease, comprising administering to the patient an effective amount of any of the above-mentioned antibodies. In some methods, the disease is Parkinson's disease. In some methods, decline of cognitive function in the patient is inhibited. In some methods, neuritic and/or axonal alpha synuclein aggregates are reduced. In some methods, neuritic dystrophy in the patient is reduced. In some methods, synaptic and/or dendritic density is preserved. In some methods, the method preserves synaptophysin and/or MAP2 in the patient.

[0015] The disclosure further provides methods of detecting Lewy bodies in a patient having or at risk of a Lewy body disease, comprising administering to the patient an effective amount of any of the above-mentioned antibodies, wherein the antibody binds to Lewy bodies and bound antibody is detected. In some methods, the disease is Parkinson's disease. In some methods, the antibody is labeled.

[0016] The invention further provides a method of producing an antibody, comprising culturing cells transformed with nucleic acids encoding the heavy and light chains of the antibody, so that the cell secrete the antibody; and purifying the antibody from cell culture media; wherein the antibody is any of the antibodies described above.

[0017] The invention further provides a method producing a cell line producing an antibody, comprising introducing a vector encoding heavy and light chains of an antibody and a selectable marker into cells; propagating the cells under conditions to select for cells having increased copy number of the vector; isolating single cells from the selected cell; and banking cells cloned from a single cell selected based on yield of antibody; wherein the antibody is any of the antibodies described above. Some such methods further comprises propagating the cells under selective conditions and screening for cell lines naturally expressing and secreting at least 100 mg/L/10^6 cells/24 h.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018]

FIG. 1 shows an alignment of the amino acid sequences of m1H7 with four versions the humanized 1H7 heavy chain mature variable region. BAC02037 (SEQ ID NO:42) is human acceptor V_H sequence. CDR regions according to Kabat definition are underlined and in bold.

FIG. 2 shows an alignment of the amino acid sequences of m1H7 with four versions the humanized 1H7 light chain mature variable region. AAY33358 (SEQ ID NO:43) is human acceptor V_L sequence. CDR regions according to Kabat definition are underlined and in bold.

FIGS. 3 A-C shows Biacore binding kinetic analysis of murine 1H7 (A), chimeric 1H7 (B) and humanized 1H7 Hu1H7VHv3-Hu1H7VLv3, respectively.

FIGS. 4 A-C shows binding kinetic parameters (ForteBio) humanized 1H7 (Hu1H7VHv2-Hu1H7VLv4, Hu1H7VHv3-Hu1H7VLv1, Hu1H7VHv3-Hu1H7VLv2, Hu1H7VHv3-Hu1H7VLv3, Hu1H7VHv3-Hu1H7VLv4, Hu1H7VHv4-Hu1H7VLv1) and chimeric 1H7.

FIG. 5 shows the results of passive immunotherapy with 1H7 on memory performance in the Morris water maze test.

FIG. 6 shows the results of passive immunotherapy with 1H7 on speed and errors in the round beam test.

BRIEF DESCRIPTION OF THE SEQUENCES

[0019]

SEQ ID NO:1 is the natural human wildtype alpha-synuclein amino acid sequence.

SEQ ID NO:2 is the non-amyloid component (NAC) domain of alpha-synuclein as reported by Jensen et al. (Biochem.

J. 310 (Pt 1): 91-94, 1995; GenBank accession number S56746).

SEQ ID NO:3 is the non-amyloid component (NAC) domain of alpha-synuclein as reported by Ueda et al. (Proc. Natl. Acad. Sci. USA, 90:11282-6, 1993).

SEQ ID NO:4 is the murine 1H7 antibody(m1H7) heavy chain variable nucleotide sequence.

SEQ ID NO:5 is the m1H7 heavy chain variable amino acid sequence.

SEQ ID NO:6 is the m1H7 light chain variable nucleotide sequence.

SEQ ID NO:7 is the m1H7 light chain variable amino acid sequence.

SEQ ID NO:8 is the mature m1H7 heavy chain variable nucleotide sequence.

SEQ ID NO:9 is the mature m1H7 heavy chain variable amino acid sequence.

SEQ ID NO:10 is the mature m1H7 light chain variable nucleotide sequence.

SEQ ID NO:11 is the mature m1H7 light chain variable amino acid sequence.

SEQ ID NO:12 is the m1H7 heavy chain CDR1 (Kabat definition).

SEQ ID NO:13 is the m1H7 heavy chain CDR2 (Kabat definition).

SEQ ID NO:14 is the m1H7 heavy chain CDR3 (Kabat definition).

SEQ ID NO:15 the m1H7 light chain CDR1 (Kabat definition).

SEQ ID NO:16 is the m1H7 light chain CDR2 (Kabat definition).

SEQ ID NO:17 is the m1H7 light chain CDR3 (Kabat definition).

SEQ ID NO:18 is the Hu1H7VHv1 nucleic acid sequence.

SEQ ID NO:19 is the Hu1H7VHv1 amino acid sequence.

SEQ ID NO:20 is the Hu1H7VHv2 nucleic acid sequence.

SEQ ID NO:21 is the Hu1H7VHv2 amino acid sequence.

SEQ ID NO:22 is the Hu1H7VHv3 nucleic acid sequence.

SEQ ID NO:23 is the Hu1H7VHv3 amino acid sequence.

SEQ ID NO:24 is the Hu1H7VHv4 nucleic acid sequence.

SEQ ID NO:25 is the Hu1H7VHv4 amino acid sequence.

SEQ ID NO:26 is the Hu1H7VHv5 nucleic acid sequence.

SEQ ID NO:27 is the Hu1H7VHv5 amino acid sequence.

SEQ ID NO:28 is the Hu1H7VH signal peptide nucleic acid sequence.

SEQ ID NO:29 is the Hu1H7VH signal peptide amino acid sequence.

SEQ ID NO:30 is the Hu1H7VH signal peptide nucleic acid sequence.

SEQ ID NO:31 is the Hu1H7VH signal peptide amino acid sequence.

SEQ ID NO:32 is the Hu1H7VLv1 nucleic acid sequence.

SEQ ID NO:33 is the Hu1H7VLv1 amino acid sequence.

SEQ ID NO:34 is the Hu1H7VLv2 nucleic acid sequence.

SEQ ID NO:35 is the Hu1H7VLv2 amino acid sequence.

SEQ ID NO:36 is the Hu1H7VLv3 nucleic acid sequence.

SEQ ID NO:37 is the Hu1H7VLv3 amino acid sequence.

SEQ ID NO:39 is the Hu1H7VLv4 amino acid sequence.

SEQ ID NO:40 is the Hu1H7VL signal peptide nucleic acid sequence.

SEQ ID NO:41 is the Hu1H7VL signal peptide amino acid sequence.

SEQ ID NO:42 is the BAC02037 (GI-21670055) human acceptor used for heavy chain framework Amino acid sequence.

SEQ ID NO:43 is the AAY33358 (GI-63102905) human acceptor used for light chain framework amino acid sequence.

SEQ ID NO:44 is the Hu1H7VH having no backmutation or CDR mutation.

SEQ ID NO:45 is the Hu1H7VLhaving no backmutation or CDR mutation.

SEQ ID NO:46 is the sequence for Hu1H7VH alternatives.

SEQ ID NO:47 is the sequence for Hu1H7VL alternatives.

SEQ ID NO:48 is the sequence for Hu1H7VH CDR3 alternatives.

SEQ ID NO:49 is the Hu1H7 light chain constant region (with arginine) (common for v1-v4).

SEQ ID NO:50 is the Hu1H7 heavy chain constant region (IgG1; common for v1-v5).

SEQ ID NO:51 is the Hu1H7 light chain constant region (without arginine) (common for v1-v4).

SEQ ID NO:52 is the Hu1H7 heavy chain constant region (Glm3 allotype).

SEQ ID NO:53 is the Hu1H7 light chain version 3 (variable region + constant region with arginine).

SEQ ID NO:54 is the Hu1H7 light chain version 3 (variable region + constant region without arginine).

SEQ ID NO:54 is the Hu1H7 heavy chain version 3 (variable region + constant region).

SEQ ID NO:56 is the Hu1H7 heavy chain version 3 (variable region + constant region; Glm3 allotype).

SEQ ID NO:57 is the Hu1H7 heavy chain constant region (IgG2).

SEQ ID NO:58 is the Hu1H7 heavy chain constant region (Glml allotype).

DEFINITIONS

[0020] Monoclonal antibodies are typically provided in isolated form. This means that an antibody is typically at least

50% w/w pure of proteins and other macromolecules arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutical acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95 or 99% w/w pure of proteins and other macromolecules from production or purification.

[0021] Specific binding of a monoclonal antibody to its target antigen means an affinity of at least 10^6, 10^7, 10^8, 10^9, or 10^{10} M^{-1}. Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (e.g., lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that a monoclonal antibody binds one and only one target.

[0022] The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region means a light chain variable region without the light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

[0023] Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. (See generally, Fundamental Immunology (Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7).

[0024] The mature variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. The CDRs from the two chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the definitions of Kabat, Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD, 1987 and 1991), or Chothia & Lesk, J. Mol. Biol. 196:901-917 (1987); Chothia et al., Nature 342:878-883 (1989). Kabat also provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number (e.g., H83 means position 83 by Kabat numbering in the mature heavy chain variable region; likewise position L36 means position 36 by Kabat numbering in the mature light chain variable region). Kabat numbering is used throughout in referring to positions in the variable region of an antibody unless explicitly stated otherwise.

[0025] The term "antibody" includes intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', $F(ab')_2$, F(ab)c, diabodies, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term "antibody" also includes a bispecific antibody and/or a humanized antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites (see, e.g., Songsivilai and Lachmann, Clin. Exp. Immunol., 79:315-321 (1990); Kostelny et al., J. Immunol. 148:1547-53 (1992)). In some bispecific antibodies, the two different heavy/light chain pairs include a humanized 1H7 heavy chain/light chain pair and a heavy chain/light chain pair specific for a different epitope on alpha synuclein than that bound by 1H7.

[0026] In some bispecific antibodies, one heavy chain light chain pair is a humanized 1H7 antibody as further disclosed below and the heavy light chain pair is from an antibody that binds to a receptor expressed on the blood brain barrier, such as an insulin receptor, an insulin-like growth factor (IGF) receptor, a leptin receptor, or a lipoprotein receptor, or a transferrin receptor (Friden et al., PNAS 88:4771-4775, 1991; Friden et al., Science 259:373-377, 1993). Such a bispecific antibody can be transferred cross the blood brain barrier by receptor-mediated transcytosis. Brain uptake of the bispecific antibody can be further enhanced by engineering the bi-specific antibody to reduce its affinity to the blood brain barrier receptor. Reduced affinity for the receptor resulted in a broader distributioin in the brain (see, e.g., Atwal. et al. Sci. Trans. Med. 3, 84ra43, 2011; Yu et al. Sci. Trans. Med. 3, 84ra44, 2011).

[0027] Exemplary bispecific antibodies can also be (1) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu et al., Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig™) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (2) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (3) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (4) a so called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting

of two identical Fab fragments linked to a different Fab fragment; (5) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fc-region. Examples of platforms useful for preparing bispecific antibodies include but are not limited to BiTE (Micromet), DART (MacroGenics), Fcab and Mab2 (F-star), Fc-engineered IgG1 (Xencor) or DuoBody (based on Fab arm exchange, Genmab).

[0028] The term "epitope" refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, *e.g.*, Epitope Mapping Protocols, in Methods in Molecular Biology, Vol. 66, Glenn E. Morris, Ed. (1996).

[0029] Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined by X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

[0030] Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (see, e.g., Junghans et al., Cancer Res. 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (e.g., at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50%, for example, 75%, 90% or 99% as measured in a competitive binding assay. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

[0031] A "patient" includes a human or other mammalian subject that receive either prophylactic or therapeutic treatment.

[0032] For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

[0033] Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0034] Compositions or methods "comprising" one or more recited elements may include other elements not specifically recited. For example, a composition that comprises antibody may contain the antibody alone or in combination with other ingredients.

[0035] Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

[0036] Unless otherwise apparent from the context, the term "about" encompasses values within a standard margin of error of measurement (SEM) of a stated value.

[0037] An individual is at increased risk of a disease if the subject has at least one known risk-factor (e.g., genetic, biochemical, family history, situational exposure) placing individuals with that risk factor at a statistically significant greater risk of developing the disease than individuals without the risk factor.

[0038] The term "symptom" refers to a subjective evidence of a disease, such as altered gait, as perceived by the patient. A "sign" refers to objective evidence of a disease as observed by a physician.

[0039] Statistical significance means $p \leq 0.05$.

[0040] "Cognitive function" refers to mental processes such as any or all of attention, memory, producing and understanding language, solving problems, and making an interest in one's surroundings and self-care.

[0041] "Enhanced cognitive function" or "improved cognitive function" refers to improvement relative to a baseline, for example, diagnosis or initiation of treatment. "Decline of cognitive function" refers to a decrease in function relative to such a base line.

[0042] In animal model systems such as rat or mouse, cognitive function may be measured methods including using a maze in which subjects use spatial information (e.g., Morris water maze, Barnes circular maze, elevated radial arm

maze, T maze and others), fear conditioning, active avoidance, illuminated open-field, dark activity meter, elevated plus-maze, two-compartment exploratory test or forced swimming test.

[0043] In humans, cognitive function can be measured by one or more of several standardized tests. Examples of a test or assay for cognitive function were described (Ruoppila, 1. and Suutama, T. Scand. J. Soc. Med. Suppl. 53,44-65, 1997) and include standardized psychometric tests (e. g. Wechsler Memory Scale, the Wechsler Adult Intelligence Scale, Raven's Standard Progressive Matrices, Schaie-Thurstone Adult Mental Abilities Test), neuropsychological tests (e. g. Luria-Nebraska), metacognitive self-evaluations (e. g. Metamemory Questionnaire), visual-spatial screening tests (e. g. Poppelreuter's Figures, Clock Recognition, Honeycomb Drawing and Cancellation), cognitive screening tests (e. g. Folstein's Mini Mental State Test) and reaction time tests. Other standard tests for cognitive performance include the Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog); the clinical global impression of change scale (CIBIC-plus scale); the Alzheimer's Disease Cooperative Study Activities of Daily Living Scale (ADCS-ADL); the Mini Mental State Exam (MMSE); the Neuropsychiatric Inventory (NPI); the Clinical Dementia Rating Scale (CDR); the Cambridge Neuropsychological Test Automated Battery (CANTAB) or the Sandoz Clinical Assessment- Geriatric (SCAG), Stroop Test, Trail Making, Wechsler Digit Span, and the CogState computerized cognitive test. In addition, cognitive function may be measured using imaging techniques such as Positron Emission Tomography (PET), functional magnetic resonance imaging (fMRI), Single Photon Emission Computed Tomography (SPECT), or any other imaging technique that allows one to measure brain function.

DETAILED DESCRIPTION OF THE INVENTION

I. GENERAL

[0044] The invention provides humanized 1H7 antibodies (Hu1H7 antibodies) as claimed. The antibodies are useful for treatment and diagnoses of a Lewy body disease.

II. TARGET MOLECULES

[0045] Natural human wildtype alpha-synuclein is a peptide of 140 amino acids having the following amino acid sequence:

MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKTK EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP DNEAYEMPSE EGYQDYEPEA (SEQ ID NO:1)

(Ueda et al., Proc. Natl. Acad. Sci. USA, 90:11282-6, 1993; GenBank accession number: P37840). The protein has three recognized domains, a KTKE repeat domain covering amino acids 1-61, a NAC (Non-amyloid component) domain running from about amino acids 60-95, and a C-terminal acidic domain running from about amino acid 98 to 140. Jensen et al. have reported NAC has the amino acid sequence: EQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFV (SEQ ID NO: 2) (Jensen et al., Biochem. J. 310 (Pt 1): 91-94, 1995; GenBank accession number S56746). Ueda et al. have reported NAC has the amino acid sequence: KEQVTNVGGAVVTGVTAVAQKTVEGAGS (SEQ ID NO: 3) (Ueda et al., Proc. Natl. Acad. Sci. USA, 90:11282-6,1993).

[0046] Unless otherwise apparent from the context, reference to alpha-synuclein or its fragments includes the natural human wildtype amino acid sequences indicated above, and human allelic variants thereof, particularly those associated with Lewy body disease (e.g., E46K, A30P and A53T, with the first letter indicates the amino acid in SEQ ID NO:1, the number is the codon position in SEQ ID NO:1, and the second letter is the amino acid in the allelic variant). Such variants can optionally be present individually or in any combination. The induced mutations E83Q, A90V, A76T, which enhance alpha synuclein aggregation, can also be present individually or in combination with each other and/or human allelic variants E46K, A30P and A53T.

III. LEWY BODY DISEASES

[0047] Lewy Body Diseases (LBD) are characterized by degeneration of the dopaminergic system, motor alterations, cognitive impairment, and formation of Lewy bodies (LBs). (McKeith et al., Neurology (1996) 47:1113-24). Lewy Bodies are spherical protein deposits found in nerve cells. Their presence in the brain disrupts the brain's normal function interrupting the action of chemical messengers including acetylcholine and dopamine. Lewy Body diseases include Parkinson's disease (including idiopathic Parkinson's disease), Diffuse Lewy Body Disease (DLBD) also known as

Dementia with Lewy Bodies (DLB), Lewy body variant of Alzheimer's disease (LBV), Combined Alzheimer's and Parkinson disease and as multiple system atrophy (MSA; *e.g.*, Olivopontocerebellar Atrophy, Striatonigral Degeneration and Shy-Drager Syndrome). DLBD shares symptoms of both Alzheimer's and Parkinson's disease. DLBD differs from Parkinson's disease mainly in the location of Lewy Bodies. In DLBD Lewy Bodies form mainly in the cortex. In Parkinson's disease, they form mainly in the substantia nigra. Other Lewy Body diseases include Pure Autonomic Failure, Lewy body dysphagia, Incidental LBD, and Inherited LBD (e.g., mutations of the alpha-synuclein gene, PARK3 and PARK4).

IV. ANTIBODIES

A. Binding Specificity and Functional Properties

[0048] Humanized antibodies of the disclosure specifically bind to human alpha synuclein. The affinity of some humanized antibodies (i.e., Ka) is can be, for example, within a factor of five or two of that of the mouse antibody (m1H7). Some humanized antibodies have an affinity that is the same, within SEM, as m1H7. Some humanized antibodies have an affinity greater than that of mouse 1H7. Some disclosed humanized antibodies bind to the same epitope and/or compete with m1H7 for binding to human alpha synuclein.

B. Humanized Antibodies

[0049] A humanized antibody is a genetically engineered antibody in which the CDRs from a non-human "donor" antibody are grafted into human "acceptor" antibody sequences (see, e.g., Queen et al., US 5,530,101 and 5,585,089; Winter et al., US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205 and 6,881,557; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody variable region sequence, a composite of such sequences, a consensus sequence of human antibody variable region sequences (e.g., light and heavy chain variable region consensus sequences of Kabat, 1991, supra), or a germline variable region sequence.

[0050] An example of an acceptor sequence for the heavy chain is the human mature heavy chain variable region with NCBI accession code BAC02037 (GI: 21670055). This acceptor sequence includes two CDRs having the same canonical form as mouse 1H7 heavy chain and has a sequence identity of 65.8% in the heavy chain variable region framework. If a different acceptor sequence is used, such an acceptor is can be, for example, another mature heavy chain variable region derived from germline VH1-18 or a mature heavy chain variable region sequence incorporating one of these germ line sequences.

[0051] For the light chain, an example of an acceptor sequence is the light chain mature variable region with NCBI accession code AAY33358 (GI:63102905). This acceptor sequence includes two CDRs having the same canonical form as a mouse 1H7 light chain and has a sequence identity of 65.4% in the light chain variable region framework. If a different acceptor is used, such an acceptor is preferably another mature light chain sequence derived from the germline A30 or a light chain mature variable region sequence incorporating one of these germ line sequences.

[0052] A humanized antibody of the disclosure is an antibody having three light chain and three heavy chain CDRs as defined by Kabat entirely or substantially from the donor mouse 1H7 antibody and mature variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Likewise a humanized heavy chain is a heavy chain having three heavy chain CDRs as defined by Kabat entirely or substantially from the heavy chain of the mouse 1H7 antibody, and a mature heavy chain variable sequence and heavy chain constant region sequence, if present, entirely or substantially from human antibody heavy chain sequence. Likewise a humanized light chain is a light chain having three light chain CDRs as defined by Kabat entirely or substantially from the light chain of the m1H7 antibody, and a mature light chain variable sequence and light chain constant region sequence, if present, entirely or substantially from human antibody light chain sequence. A CDR is substantially from m1H7 if at least 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of residues are identical to the corresponding residues in the corresponding CDR of m1H7. The mature variable region framework sequences of an antibody chain or the constant region sequence of an antibody chain are substantially from a human mature variable region framework sequence or human constant region sequence respectively when at least 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% of corresponding residues defined by Kabat are identical.

[0053] Certain amino acids from the human mature variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen, mediating interaction between heavy and light chains, interaction with the constant region, being a site for desired or undesired post-translational modification, being an unusual residue for its position in a human variable region sequence and therefore potentially immunogenic, among other reasons. The following 11 variable region framework positions were considered as candidates for substitutions for one or more of these reasons as further specified in the Examples (L46F, Y49C, F83A, V11L, T28S, R38K, M48I, V67A, M69L, T71A, Y91F).

[0054] Here as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat

CDRs into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus within variable region frameworks, the first mentioned residue is human and within CDRs the first mentioned residue is mouse (e.g., C97S).

[0055] Amino acid substitutions can be made in the CDRs. One possible variation is to substitute certain residues in the CDRs of the mouse 1H7 antibody with corresponding residues from human CDRs sequences, typically from the CDRs of the human acceptor sequences used in designing the exemplified humanized antibodies. In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, J. Mol. Biol. 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales et al., Mol. Immunol. 41:863 (2004). In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor 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.

[0056] One reason for performing a substitution within a CDR is that a mouse residue is a site of posttranslational modification that may interfere with expression or assembly of an antibody. Here, position H97 within CDRH3, which is occupied by a C in mouse 1H7 was identified as a site for substitution.

[0057] The 11 variable region framework backmutations and 1 CDR substitution noted above can be incorporated into humanized 1H7 antibodies in many permutations. The heavy chain variable region of such antibodies can be represented by a sequence comprising QVQLVQSGAE-X_1-KKPGASVKVSCKASGY-X_2-FTSYYIHWV-X_3-QAPGQGLEW-X_4-GWI-YPGSGNTKYSEKFKGR-X_5-T-X_6-T-X_7-DTSTSTAYMELRSLRSDDTAVY-X_8-CARDG-X_9-YGFAYWGQGTLVTVSS, wherein -X_1- is V or L; -X_2- is S or T; -X_3- is R or K; -X_4- is M or I; -X_5- is V or A; -X_6- is M or L; -X_7- is T or A; -X_8- is Y or F; -X_9- is C, M, S, or T (SEQ ID NO:46). In some heavy chain variable regions, -X_9- is C. Some light chain variable regions can be represented by a sequence comprising DIQLTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMN-WYQQKPGKAPK-Z_1-LI-Z_2-AASNLESGVPSRFSGSGSGTDFTLTISSLQPED-Z_3-ATYYCQQSNEDPFTFGQGTK-LEIK, wherein -Z_1- is L or F; -Z_2- is Y or C; -Z_3- is F or A (SEQ ID NO:47). In some disclosed antibodies the heavy chain variable region comprises SEQ ID NO: 46 and the light chain variable region comprises SEQ ID NO:47. For example, residues, X_1 and X_3 of SEQ ID NO: 48 are V and R, respectively. For example, residues, X_5-and X_7 of SEQ ID NO:46 are A and residueZ_1 of SEQ ID NO:47 is F. For example, residues, -X_4, X_6 , and X_8 of SEQ ID NO:46 are M, M, and Y, respectively, and residue Z_2 and $_3$ of SEQ ID NO:47 are C and F, respectively. For example, residues, X_2 and X_9 of SEQ ID NO: 48 are T and C, respectively.

[0058] Some disclosed antibodies contain two heavy chain substitutions and two light chain substitutions. For example, position H67 is occupied by A, H71 is occupied by A, position L46 is occupied by F, and position L49 is occupied by C. In some antibodies, the heavy chain mature variable region has an amino acid sequence designated SEQ ID NO:23. In some antibodies, the light chain mature variable region has an amino acid sequence designated SEQ ID NO:37. For example, in H3L3 (Hu1H7VHv3 (SEQ ID NO:23-Hu1H7VLv3 (SEQ ID NO:37)), the heavy chain mature variable region has an amino acid sequence designated SEQ ID NO:23, and the light chain mature variable region has an amino acid sequence designated SEQ ID NO:37.

[0059] The disclosure provides variants of the H3L3 humanized antibody in which the humanized heavy chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% or 99% identity to SEQ ID NO:23 and the humanized light chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% or 99% sequence identity to SEQ ID NO:37. Some such humanized antibodies include three heavy and three light chain CDRs entirely or substantially identical to the CDR regions of H3L3, which are the same as those of the mouse donor antibody. The CDR regions can be defined by any conventional definition (e.g., Chothia) but are preferably as defined by Kabat.

[0060] Some variants of the H3L3 humanized antibody retain some or all of the backmutations in H3L3. In other words, at least 1, 2, 3 or preferably all 4 of the following are present: H67 is occupied by A, and H71 is occupied by A, L46 is occupied by F, and position L49 is occupied by C.

[0061] In addition to retaining at least 1, 2, 3 or preferably all 4 of the backmutations of H3L3, humanized 1H7 antibodies may also contain additional backmutations in the variable region frameworks. Examples of such backmutations include H11 occupied by L, H28 occupied by S, H38 occupied by K, H48 occupied by I, H69 occupied by L, H91 occupied by F, and/or L83 occupied by A. For selection of backmutations for a therapeutic or diagnostic product, one should take into account the degree to which they in general do not improve affinity and the degree to which introducing more mouse residues may give increased risk of immunogenicity. For example, H3L1 comprises a heavy chain mature variable region of SEQ ID NO:23, and a light chain of SEQ ID NO:33. For example, H4L1 comprises a heavy chain mature variable

region of SEQ ID NO:25, and a light chain of SEQ ID NO:33.

[0062] Another possibility for variation is to use a different human acceptor sequences as discusseed above. Substitutions in CDR regions are possible as described above, for example at position H97, but prior to selecting such substitutions for a therapeutic or diagnostic product, one should consider the potential effect on affinity and antibody expression.

[0063] If position H97 in mouse CDRH3 is other than cysteine, it is preferably occupied by M, S, or T. Some disclosed antibodies comprise a humanized heavy chain comprising Kabat CDR1 of SEQ ID NO:12: SYYIH; Kabat CDR2 of SEQ ID NO:13: WIYPGSGNTKYSEKFKG; Kabat CDR3 of SEQ ID NO:48: DG-X_9-YGFAY, wherein -X_9-is C, M, S, or T, more preferably C. Some antibodies comprise a humanized light chain comprising Kabat CDR1 of SEQ ID NO: 15: KASQSVDYDGDSYMN; Kabat CDR2 of SEQ ID NO:16: AASNLES; Kabat CDR3 of SEQ ID NO:17: QQSNEDPFT. Some antibodies comprise a humanized heavy chain comprising the three Kabat CDRs of SEQ ID NOs:12, 13, and 48 and a humanized light chain comprising the three Kabat CDRs of SEQ ID NOs:15-17. In some such antibodies, a humanized heavy chain comprises the three Kabat CDRs of SEQ ID NO:12-14 and a humanized light chain comprises the three Kabat CDRs of SEQ ID NO:15-17.

[0064] The disclosure further provides humanized 1H7 antibodies in which the humanized heavy chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% , or 99% identity or is 100% identity with SEQ ID NOs. 19, 21, 23, 25, and 27 and the humanized light chain mature variable region shows at least 90%, 95%, 96%, 97%, 98% , or 99% sequence identity to or is 100% identical with any one of SEQ ID NOs. 33, 35, 37, and 39.

[0065] Various permutations of the 11 variable region framework backmutations and 1 CDR mutation described above can be incorporated into the humanized 1H7 antibodies. In some such antibodies, position L46 is occupied by L or F (preferably F) and/or position L49 is occupied by Y or C (preferably C) and/or position L83 is occupied by F or A , and/or position H11 is occupied by V or L, and/or position H28 is occupied by T or S, and/or position H38 is occupied by R or K, and/or position H48 is occupied by M or I, and/or position H67 is occupied by V or A (preferably A), and/or position H69 is occupied by M or L, and/or position H71 is occupied by T or A (preferably A), and/or position H91 is occupied by Y or F, and/or position H28 is occupied by S or T, and/or position H97 is occupied by C or M or S or T (preferably C).

[0066] In some such antibodies, some or all of the backmutations in Hu1H7VLv1-v4 and Hu1H7VHv1-v5 are retained. Preferably, the backmutations at positions L46, H67, and H71 are retained. In other words, position L46 is occupied by F, position H67 is occupied by A, and position H71 is occupied by A. More preferably, the backmutations at positions L46, L49, H67, and H71 are retained. In other words, position L46 is occupied by F, position L49 is occupied by C, position H67 is occupied by A, and position H71 is occupied by A. In some antibodies, some or all of heavy chain positions H11, H28, H38, H48, H67, H69, H71, and/or H91 are occupied by L, S, K, I, A, L, A, and F respectively. Likewise in some antibodies some or all of light chain positions L46, L49 and/or L83 are occupied by F, C and A respectively. In some antibodies, 1, 2, 3, 4, 5, 6, 7, 8, 9 or all ten of positions H11, H28, H38, H48, H67, H69, H71, H91, L46, L49 and L83 is/are occupied by L, S, K, I, A, L, A, F, F, C and A respectively. In some antibodies, 0, 1, 2, 3, 4, 5, 6, or 7 positions are changed in the heavy chain mature variable region framework relative to SEQ ID NO:44, and 0, 1, 2, or 3 positions are change in the light chain mature variable region framework relative to SEQ ID NO:45. In some such antibodies, position H97 is occupied by C. Preferably, the humanized antibody has a K_D for alpha-synuclein from about 0.5 to 2 of that of a murine or chimeric 1H7 antibody.

[0067] In some disclosed antibodies, position L46 is occupied by F, position H67 is occupied by A, position H71 is occupied by A, position H11 is occupied by V, and position H38 is occupied by R. In some such antibodies, position L49 is occupied by Y. More preferably, in some such antibodies, position L49 is occupied by C. In some such antibodies, position L83 is occupied by F. In some such antibodies, position L83 is occupied by A. In some such antibodies, position H97 is occupied by C. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by M, position H69 is occupied by M and position H91 is occupied by Y. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by M, position H69 is occupied by M and position H91 is occupied by F. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by M, position H69 is occupied by L and position H91 is occupied by Y. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by M, position H69 is occupied by L and position H91 is occupied by F. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by I, position H69 is occupied by M and position H91 is occupied by Y. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by I, position H69 is occupied by M and position H91 is occupied by F. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by I, position H69 is occupied by L and position H91 is occupied by Y. In some such antibodies, position H28 is occupied by T or S, position H48 is occupied by I, position H69 is occupied by L and position H91 is occupied by F. In some such antibodies, position H11 is occupied by L, and/or position H38 is occupied by K, and/or position H97 is occupied by S.

[0068] In any of the above disclosed antibodies, other amino acid substitutions can be made in the mature variable region framework, for example, in residues not in contact with the CDRs. Often the replacements made in the variant humanized sequences are conservative with respect to the replaced amino acids. In some antibodies, replacements relative to Hu1H7VLv1-v4 and Hu1H7VHv1-v5 (whether or not conservative) have no substantial effect on the binding affinity or potency of the resultant antibody relative to Hu1H7VLv1-v4 and Hu1H7VHv1-v5, that is, its ability to bind

human alpha synuclein.

[0069] Variants typically differ from the heavy and light chain mature variable region sequences of Hu1H7VLv1-v4 and Hu1H7VHv1-v5 by a small number (e.g., typically no more than 1, 2, 3, 5 or 10 in either the light chain or heavy chain mature variable region framework, or both) of replacements, deletions or insertions.

C. Selection of Constant Region

[0070] The heavy and light chain variable regions of humanized antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotopes IgG1 and IgG3 have complement-dependent cytotoxicity and human isotypes IgG2 and IgG4 do not. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa. An exemplary human light chain kappa constant region has the amino acid sequence of SEQ ID NO:49. The N-terminal arginine of SEQ ID NO:49 can be omitted, in which case light chain kappa constant region has the amino acid sequence of SEQ ID NO:51. An exemplary human IgG1 heavy chain constant region has the amino acid sequence of SEQ ID NO:50. An exemplary human IgG2 heavy chain constant region has the amino acid sequence of SEQ ID NO:57. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')2, and Fv, or as single chain antibodies in which heavy and light chain mature variable domains are linked through a spacer.

[0071] Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype bind to a non-polymorphic region of a one or more other isotypes. Thus, for example, heavy chain constant regions can be of IgG1 G1m1 or IgG1 G1m3 allotypes and have the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:55. Yet another heavy chain constant region has the amino acid sequence of SEQ ID NO:52 or SEQ ID NO:55 except that it lacks the C-terminal lysine.

[0072] One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (see, e.g., Winter et al., US Patent No. 5,624,821; Tso et al., US Patent No. 5,834,597; and Lazar et al., Proc. Natl. Acad. Sci. USA 103:4005, 2006), or to prolong half-life in humans (see, e.g., Hinton et al., J. Biol. Chem. 279:6213, 2004). Exemplary substitutions include a Gln at position 250 and/or a Leu at position 428 (EU numbering is used in this paragraph for the constant region) for increasing the half life of an antibody. Substitution at any or all of positions 234, 235, 236 and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor (see, e.g., US 6,624,821). Some antibodies have alanine substitution at positions 234, 235 and 237 of human IgG1 for reducing effector functions. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine (see, e.g., US 5,624,821).

[0073] In some antibodies, the light chain constant region has the amino acid sequence designated SEQ ID NO:49. In some antibodies, the heavy chain constant region has the amino acid sequence designated SEQ ID NO:52. Exemplary Hu1H7 light chains have SEQ ID NO:53 or SEQ ID NO:54. Exemplary Hu1H7 heavy chains have the amino acid sequence of SEQ ID NO:55 or SEQ ID NO:56. In some antibodies, the light chain is SEQ ID NO:53. In some antibodies, the heavy chain is SEQ ID NO:56.

D. Expression of Recombinant Antibodies

[0074] Antibodies can be produced by recombinant expression. Nucleic acids encoding the antibodies can be codon-optimized for expression in the desired cell-type (e.g., CHO or Sp2/0). Recombinant nucleic acid constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. The expression control sequences can be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification of the crossreacting antibodies. The vector or vectors encoding the antibody chains can also contain a selectable gene, such as dihydrofolate reductase, to allow amplification of copy number of the nucleic acids encoding the antibody chains.

[0075] E. coli is a prokaryotic host particularly useful for expressing antibodies, particularly antibody fragments. Microbes, such as yeast are also useful for expression. Saccharomyces is an example of a yeast host, with suitable vectors having expression control sequences, an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose

utilizations.

[0076] Mammalian cells can be used for expressing nucleotide segments encoding immunoglobulins or fragments thereof. See Winnacker, From Genes to Clones, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. It can be advantageous to use nonhuman cells. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen et al., Immunol. Rev. 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Suitable expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. See Co et al., J. Immunol. 148:1149 (1992).

[0077] Having introduced vector(s) encoding antibody heavy and light chains into cell culture, cell pools can be screened for growth productivity and product quality in serum-free media. Top-producing cell pools can then be subjected to FACS-based single-cell cloning to generate monoclonal lines. Specific productivities above 50 pg or 100 pg per cell per day, which correspond to product titers of greater than 7.5 g/L culture, can be advantageous. Antibodies produced by single cell clones can also be tested for turbidity, filtration properties, PAGE, IEF, UV scan, HP-SEC, carboydrate-oligosaccharide mapping, mass spectrometery, and binding assay, such as ELISA or Biacore. A selected clone can then be banked in multiple vials and stored frozen for subsequent use.

[0078] Once expressed, antibodies can be purified according to standard procedures of the art, including protein A capture, column chromatography (e.g., hydrophobic interaction or ion exchange), low-pH for viral inactivation and the like (see generally, Scopes, Protein Purification (Springer-Verlag, NY, 1982)).

[0079] Methodology for commercial production of antibodies including codon optimization, selection of promoters, transcription elements, and terminators, serum-free single cell cloning, cell banking, use of selection markers for amplification of copy number, CHO terminator, serum free single cell cloning, improvement of protein titers (see, e.g., US 5,786,464, US 5,888,809, US 6,063,598, US 6,114,148, US 7,569,339, WO2004/050884, WO2005/019442, WO2008/012142, WO2008/012142, WO2008/107388, and WO2009/027471, and).

E. Nucleic Acids

[0080] The disclosure further provides nucleic acids encoding any of the heavy and light chains described above. Typically, the nucleic acids also encode a signal peptide fused to the mature heavy and light chains (e.g., signal peptides having amino acid sequences of SEQ ID NOS: 29, 31 and 41 that can be encoded by SEQ ID NOS: 28, 30 and 40). Coding sequences on nucleic acids can be in operable linkage with regulatory sequences to ensure expression of the coding sequences, such as a promoter, enhancer, ribosome binding site, transcription termination signal and the like. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, e.g., within an expression vector, or can be separate, e.g., each cloned into its own expression vector.

V. THERAPEUTIC APPLICATIONS

[0081] The invention provides an antibody for use in several methods of treating or effecting prophylaxis of Lewy Body disease in patients suffering from or at risk of such disease. Patients amenable to treatment include individuals at risk of disease of a LBD but not showing symptoms, as well as patients presently showing symptoms or the early warning signs of synucleinopathies, for example, EEG slowing, neuropsychiatric manifestations (depression, dementia, hallucinations, anxiety, apathy, anhedonia), autonomic changes (orthostatic hypotension, bladder disturbances, constipation, fecal incontinence, sialorrhea, dysphagia, sexual dysfunction, changes in cerebral blood flow), sensory changes (olfactory, pain, color discrimination abnormal sensations), sleep disorders (REM sleep behavior disorder (RBD), restless legs syndrome/periodic extremity movements, hypersomnia, insomnia) and miscellaneous other signs and symptoms (fatigue, diplopia, blurred vision, seborrhea, weight loss/gain). Therefore, the present methods can be administered prophylactically to individuals who have a known genetic risk of a LBD. Such individuals include those having relatives who have experienced this disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk toward PD include mutations in the alpha-synuclein or Parkin, UCHLI, and CYP2D6 genes; particularly mutations at positions 30 and 53 of the alpha-synuclein gene. Individuals presently suffering from Parkinson's disease can be recognized from its clinical manifestations including resting tremor, muscular rigidity, bradykinesia and postural instability.

[0082] In asymptomatic patients, treatment can begin at any age (*e.g.*, 10, 20, 30). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying antibody, or activated T-cell or B-cell responses to a therapeutic agent

(*e.g.*, a truncated form of alpha-synuclein peptide) over time. If the response falls, a booster dosage is indicated.

[0083] Antibodies can be used for treating or effecting prophylaxis of Lewy Body disease in patients by administration under conditions that generate a beneficial therapeutic response in a patient (e.g., reduction of neuritic and/or axonal alpha synuclein aggregates, reduction of neuritic dystrophy, improving cognitive function, and/or reversing, treating or preventing cognitive decline) in the patient. In some methods, the areas of neuritic dystrophy in the neuropil of neocortex and/or basal ganglia can be reduced by on average at least 10%, 20%, 30%, or 40% in treated patients compared with a control population..

[0084] Cognitive impairment, progressive decline in cognitive function, changes in brain morphology, and changes in cerebrovascular function are commonly observed in patients suffering from or at risk of Lewy Body disease. Administration of the present antibodies can inhibit or delay decline of cognitive function in such patients.

[0085] The disclosure also provides methods of preserving or increasing synaptic density and/or dentritic density. An index of changes in synaptic or dentritic density can be measured by markers of synapse formation (synaptophysin) and/or dendrites (MAP2). In some methods, the synaptic or dentritic density can be restored to the level of synaptic or dentritic density in a healthy subject. In some methods, the mean level of synaptic or dentritic density in treated patients can be elevated by 5%, 10%, 15%, 20%, 25%, 30% or more as compared to a population of untreated control patients.

VI. PHARMACEUTICAL COMPOSITIONS AND METHODS OF TREATMENT

[0086] In prophylactic applications, an antibody or a pharmaceutical composition the same is administered to a patient susceptible to, or otherwise at risk of a disease in a regime (dose, frequency and route of administration) effective to reduce the risk, lessen the severity, or delay the onset of at least one sign or symptom of the disease. In some prophylactic applications, the regime is effective to inhibit or delay accumulation of alpha synuclein and truncated fragments in the brain, and/or inhibit or delay its toxic effects and/or inhibit/or delay development of behavioral deficits. In therapeutic applications, an antibody or agent to induce an antibody is administered to a patient suspected of, or already suffering from a Lewy body disease in a regime (dose, frequency and route of administration) effective to ameliorate or at least inhibit further deterioration of at least one sign or symptom of the disease. In some therapeutic applications, the regime is effective to reduce or at least inhibit further increase of levels of alpha synuclein and truncated fragments, associated toxicities and/or behavioral deficits.

[0087] A regime is considered therapeutically or prophylactically effective if an individual treated patient achieves an outcome more favorable than the mean outcome in a control population of comparable patients not treated by methods of the invention, or if a more favorable outcome is demonstrated in treated patients versus control patients in a controlled clinical trial (e.g., a phase II, phase II/III or phase III trial) at the $p < 0.05$ or 0.01 or even 0.001 level.

[0088] Effective doses vary depending upon many different factors, including means of administration, target site, physiological state of the patient including type of Lewy body disease, whether the patient is an ApoE carrier, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic.

[0089] An exemplary dosage range for antibodies is from about 0.01 to 5 mg/kg, and more usually 0.1 to 3 mg/kg or 0.15-2 mg/kg or 0.15-1.5 mg/kg, of patient body weight. Antibody can be administered such doses daily, on alternative days, weekly, fortnightly, monthly, quarterly, or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months.

[0090] Antibodies can be administered via a peripheral route (i.e., one in which an administered or induced antibody crosses the blood brain barrier to reach an intended site in the brain. Routes of administration include topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intrathecal, intraperitoneal, intranasal or intramuscular. Some routes for administration of antibodies are intravenous and subcutaneous. This type of injection is most typically performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection.

[0091] Pharmaceutical compositions for parenteral administration are can be sterile and substantially isotonic and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

[0092] The present regimes can be administered in combination with another agent effective in treatment or prophylaxis of the disease being treated. For example, in the case of Parkinson's disease, immunotherapy against alpha synuclein WO/2008/103472, Levodopa, dopamine agonists, COMT inhibitors, MAO-B inhibitors, Amantadine, or anticholinergic

agents can be used in combination with the present regimes.

VII. OTHER APPLICATIONS

[0093] The antibodies described above can be used for detecting alpha-synuclein in the context of clinical diagnosis or treatment or in research. The antibodies can also be sold as research reagents for laboratory research in detecting cells bearing alpha-synuclein and their response to various stimuli. In such uses, monoclonal antibodies can be labeled with fluorescent molecules, spin-labeled molecules, enzymes or radioisotypes, and can be provided in the form of kit with all the necessary reagents to perform the assay for alpha-synuclein. The antibodies can also be used to purify alpha-synuclein, e.g., by affinity chromatography.

[0094] The antibodies can be used for detecting LBs in a patient. Such methods are useful to diagnose or confirm diagnosis of PD, or other disease associated with the presence of LBs in the brain, or susceptibility thereto. For example, the methods can be used on a patient presenting with symptoms of dementia. If the patient has LBs, then the patient is likely suffering from a Lewy body disease, such as Parkinson's disease. The methods can also be used on asymptomatic patients. Presence of Lewy bodies or other abnormal deposits of alpha-synuclein indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in patients who have been previously diagnosed with a Lewy body disease.

[0095] The methods can be performed by administering an antibody and then detecting the antibody after it has bound. If desired, the clearing response can be avoided by using an antibody fragment lacking a full-length constant region, such as a Fab. In some methods, the same antibody can serve as both a treatment and diagnostic reagent.

[0096] For diagnosis (e.g., *in vivo* imaging), the antibodies can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the antibody is labeled, although in some methods, the antibody is unlabeled and a secondary labeling agent is used to bind to the antibody. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using PET or SPECT.

[0097] Diagnosis is performed by comparing the number, size and/or intensity of labeled loci to corresponding base line values. The base line values can represent the mean levels in a population of undiseased individuals. Base line values can also represent previous levels determined in the same patient. For example, base line values can be determined in a patient before beginning treatment, and measured values thereafter compared with the base line values. A decrease in values relative to base line signals a positive response to treatment.

[0098] The antibodies can be used to generate anti-idiotype antibodies. (see, e.g., Greenspan & Bona, FASEB J. 7(5) :437-444, 1989; and Nissinoff, J. Immunol. 147:2429-2438, 1991). Such anti-idiotype antibodies can be utilized in pharmacokinetics, pharmacodynamics, biodistribution studies as well as in studies of clinical human-anti-human antibody (HAHA) responses in individuals treated with the antibodies. For example, anti-idiotypic antibodies bind specifically the variable region of humanized 1H7 antibodies and therefore can be used to detect humanized 1H7 antibodies in pharmacokinetic studies and help to quantify human-anti-human antibody (HAHA) responses in treated individuals.

[0099] If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise.

EXAMPLES

Example I. Design of humanized 1H7 antibodies

[0100] The starting point or donor antibody for humanization is the mouse antibody 1H7 produced by the hybridoma having ATCC Accession No. PTA-8220 and described in U.S. Patent Application No. 11/710,248 published as US2009/0208487. The complete heavy chain variable amino acid and nucleic acid sequences of m1H7 are provided as SEQ ID NOS:4 and 5, respectively. The complete light chain variable amino acid and nucleic acid sequences of m1H7 are provided as SEQ ID NOS:6 and 7, respectively. The heavy chain variable amino acid and nucleic acid sequences of mature m1H7 are provided as SEQ ID NOS:8 and 9, respectively. The light chain variable amino acid and nucleic acid sequences of mature m1H7 are provided as SEQ ID NOS:10 and 11, respectively. The heavy chain CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:12, 13, and 14, respectively. The light chain CDR1,

CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:15, 16, and 17, respectively. Kabat numbering is used throughout in this Example.

[0101] The variable kappa (Vk) of m1H7 belongs to mouse Kabat subgroup 3 which corresponds to human Kabat subgroup 1. The variable heavy (Vh) of 1H7 belongs to mouse Kabat subgroup 5a which corresponds to human Kabat subgroup 1 (Kabat et al. Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242, 1991). The 15 residue CDR-L1 belongs to canonical class 5 (note that the Methionine at position 33 is usually Leucine in this class), the 7 residue CDR-L2 belongs to canonical class 1, the 9 residue CDR-L3 belongs to canonical class 1 in Vk (Martin & Thornton, J Mol Biol. 263:800-15, 1996). The 5 residue CDR-H1 belongs to canonical class 1, the 17 residue CDR-H2 belongs to canonical class 2 (Martin & Thornton, J Mol Biol. 263:800-15, 1996). The CDR-H3 has no canonical classes, but the 8 residue loop probably has a kinked base according to the rules of Shirai et al., FEBS Lett. 455:188-97 (1999).

[0102] The residues at the interface between the Vk and Vh domains are the ones commonly found, except that F46 in the kappa chain is usually a Leucine. This makes this position a candidate t for backmutation. A search was made over the protein sequences in the PDB database (Deshpande et al., Nucleic Acids Res. 33: D233-7, 2005) to find structures which would provide a rough structural model of 1H7. The 0.5B anti-HIV antibody has good overall sequence similarity to 1H7 Vk, retaining the same canonical structures for the loops. The NMR structure of the 0.5B anti-HIV antibody (pdb code 1QNZ; Tugarinov et al., Structure 8:385-95, 2000) was used for the Vk structure in the modeling. The anti-alpha-(2→8)-polysialic acid antibody has good overall sequence similarity to 1H7 Vh structure. It also has a CDR-H3 of a similar length with a kinked base. The structure of the anti-alpha-(2→8)-polysialic acid antibody (1PLG; Evans et al., Biochemistry 34:6737-44, 1995) has reasonable resolution (2.8A), and was used for the Vh structure in the modeling. In addition, CDRs-HI and H2 of the anti-alpha-(2→8)-polysialic acid antibody have the same canonical structures as 1H7 Vh. DeepView/Swiss-PdvViewer 3.7 (SP5) (Guex & Peitsch, Electrophoresis 18: 2714-2723, 1997) was used to model a rough structure of 1H7fv.

[0103] A search of the non-redundant protein sequence database from NCBI allowed selection of suitable human frameworks into which to graft the murine CDRs. For Vk, a human kappa light chain with NCBI accession code AAY33358 (GI:63102905; SEQ ID NO:43) (Kramer et al., Eur J Immunol. 35:2131-45, 2005) was chosen. This has the same canonical classes for CDR-L2 and L3, and belongs to human kappa germline A30, a member of Kabat human kappa subgroup 1. AAY33358 has a sequence identity of 65.4% in the light chain variable region framework to murine 1H7 light chain. For Vh, human Ig heavy chain BAC02037 (GI:21670055; SEQ ID NO:42) was chosen, belonging to human heavy germline VH1-18. It is a member of Kabat human heavy subgroup 1. It shares the canonical form of 1H7 CDR-H1 and H2, and H3 is 8 residues long with a predicted kinked base. BAC02037 has a sequence identity of 65.8% in the variable region framework to murine 1H7 heavy chain. Humanized 1H7 heavy and light chain variable sequences having no backmutations or CDR mutations are provided as SEQ ID NOS:44-45.

[0104] Four humanized light chain variable regions variants and five humanized heavy chain variable region variants were constructed containing different permutations of the above substitutions (Hu1H7VLv1-v4; SEQ ID NOs:32-39, and Hu1H7VHv1-v5; SEQ ID NOs:18-27 respectively) (Figs. 1-2 and Table 1). SEQ ID NOs. 19, 21, 23, 25, 27, 33, 35, 37, and 39 include backmutations as shown in Table 1. In addition, two humanized heavy chains (SEQ ID NOs: 25, 27) include C→S mutation at position H97 (Kabat numbering) of heavy chain CDR3 (Figs. 1-2 and Table 1). The amino acids at L46, L49, L83, H11, H28, H38, H48, H67, H69, H71, H91, and H97 in Hu1H7VLv1-v4 and Hu1H7VHv1-v5 are listed in Table 2.

[0105] The H3L3 variant (hu1H7VHv3 (SEQ ID NO:23)-Hu1H7VLv3 (SEQ ID NO:37) was found to give the lowest dissociation constant (highest association constant), the same as the mouse 1H7 within the SEM.

Table 1 V_H, V_L backmutations, and CDR mutations

V_H variant	V_H exon acceptor sequence	donor framework residues	CDR mutations
Hu1H7VHv1 (SEQ ID NO:19)	NCBI accession code BAC02037	H11, H28, H38, H48, H67, H69, H71, H91	
Hu1H7VHv2 (SEQ ID NO:21)	NCBI accession code BAC02037 (SEQ ID NO:42)	H28, H48, H67, H69, H71, H91	
Hu1H7VHv3 (SEQ ID NO:23)	NCBI accession code BAC02037 (SEQ ID NO:42)	H67, H71	
Hu1H7VHv4 (SEQ ID NO:25)	NCBI accession code BAC02037 (SEQ ID NO:42)	H28, H48, H67, H69, H71, H91	H97
Hu1H7VHv5 (SEQ ID NO:27)	NCBI accession code BAC02037 (SEQ ID NO:42)	H67, H71	H97

(continued)

V_H variant	V_H exon acceptor sequence	donor framework residues	CDR mutations
VL variant	VL exon acceptor sequence	donor framework residues	

Table 1 V_H, V_L backmutations, and CDR mutations

V_H variant	V_H exon acceptor sequence	donor framework residues	CDR mutations
Hu1H7VLv1 (SEQ ID NO: 33)	NCBI accession code AAY33358 (SEQ ID NO:43)	L46, L49, L83	
Hu1H7VLv2 (SEQ ID NO: 35)	NCBI accession code AAY33358 (SEQ ID NO:43)	L46, L83	
Hu1H7VLv3 (SEQ ID NO: 37)	NCBI accession code AAY33358 (SEQ ID NO:43)	L46, L49	
Hu1H7VLv4 (SEQ ID NO: 39)	NCBI accession code AAY33358 (SEQ ID NO:43)	L46	

Table 2. Kabat numbering of some framework residues for backmutation and CDR mutations in humanized 1H7 antibodies

	AAY33358 light chain	BAC02037 heavy chain	Mouse 1H7	Hu1H7VH1	HulH7VH2	HulH7VH3	HulH7VH4	HulH7VH5	HulH7VL1	HulH7VL2	HulH7VL3	HulH7VL4
H11	-	V	L	L	V	V	V	V	-	-	-	-
H28		T	S	S	S	T	S	T	-	-	-	-
H38	-	R	K	K	R	R	R	R	-	-	-	-
H48	-	M	I	I	I	M	I	M	-	-	-	-
H67	-	V	A	A	A	A	A	A	-	-	-	-
H69	-	M	L	L	L	M	L	M	-	-	-	-
H71	-	T	A	A	A	A	A	A	-	-	-	-
H91	-	Y	F	F	F	Y	F	Y	-	-	-	-
H97	-	G	C	C	C	C	S	S	-	-	-	-
L46	L	-	F	-	-	-	-	-	F	F	F	F
L49	Y	-	C	-	-	-	-	-	C	Y	C	Y
L83	F	-	A	-	-	-	-	-	A	A	F	F

[0106] The rationales for selection of the above positions as candidates for substitution are as follows.

[0107] L46F (here as elsewhere for framework backmutations, the first mentioned residue is the human residue and the second the mouse residue): This position is a Vk/Vh interface residues.

[0108] Y49C: Cysteine at this position is unusual in either mouse or human sequence. Occupying a position in the center of the antigen-binding site, this residue may bind antigen, or maintain the conformation of the loops.

[0109] F83A: In human framework, this position is occupied by phenylalanine, a larger amino acid. Therefore, A83 would be unusual in human frameworks. In the humanized antibody, the constant domain will be human not mouse, so the human F83 would be usual. However, this position in Vk is in close proximity to the constant domain and may interfere with packing against the constant region. Therefore, it would be interesting to backmutate it to A to see if there is a difference.

[0110] V11L: This position contacts the constant domain and may therefore alter the topography of the binding site.

[0111] T28S: This position contributes to CDR-H1 conformation, but may also bind antigen. The T→S mutation is a conservative mutation.

[0112] R38K: This position lies beneath CDR-H2, interacting with F63 in the model. The R→K mutation is a conservative mutation.

[0113] M48I: This position lies beneath F63 in CDR-H2 in the model. The M→I mutation is a conservative mutation.

[0114] V67A: This position lies underneath CDR-H2. The V→A mutation is not a conservative mutation.

[0115] M69L: This position lies beneath CDR-H2. The M→L mutation is a conservative mutation.

[0116] T71A: This position is a canonical residue for CDR-H2. The T→A mutation is not a conservative mutation.

[0117] Y91F: This position is an interface residue interacting with P44 in the light chain. The Y→F mutation is a conservative mutation.

[0118] C97S: This CDR mutation in CDRH3 avoids posttranslational modification of the cysteine.

>Hu1H7Vκ Version1

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIC**AASN LES**GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC**QQSNEDPFT**FGQGTKLEIK (SEQ ID NO: 33)

>Hu1H7Vκ Version2

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIY**AASN LES**GVPSRFSGSGSGTDFTLTISSLQPEDAATYYC**QQSNEDPFT**FGQGTKLEIK (SEQ ID NO: 35)

>Hu1H7Vκ Version3

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIC**AASN LES**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQSNEDPFT**FGQGTKLEIK (SEQ ID NO: 37)

>Hu1H7Vκ Version4

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIY**AASN LES**GVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQSNEDPFT**FGQGTKLEIK (SEQ ID NO: 39)

>Hu1H7vh Version1

QVQLVQSGAELKKPGASVKVSCKASGYSFT**SYYIH**WVKQAPGQGLEWIG**WIYPGSG**
NTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGCYGFAY**WGQGT
LVTVSS (SEQ ID NO: 19)

>Hu1H7vh Version2

QVQLVQSGAEVKKPGASVKVSCKASGYSFT**SYYIH**WVRQAPGQGLEWIG**WIYPGS**
GNTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGCYGFAY**WGQG
TLVTVSS (SEQ ID NO: 21)

>Hu1H7vh Version3

QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYIH**WVRQAPGQGLEWMG**WIYPGS**
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCAR**DGCYGFAY**WGQ
GTLVTVSS (SEQ ID NO: 23)

>Hu1H7vh Version4

QVQLVQSGAEVKKPGASVKVSCKASGYSFT**SYYIH**WVRQAPGQGLEWIG**WIYPGS**
GNTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGSYGFAY**WGQG
TLVTVSS (SEQ ID NO: 25)

>Hu1H7vh Version5

QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYIH**WVRQAPGQGLEWMG**WIYPGS**
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCAR**DGSYGFAY**WGQ
GTLVTVSS (SEQ ID NO: 27)

Example II. Binding kinetic analysis of murine, chimeric, and humanized1H7 antibodies

[0119] Binding kinetics of humanized 1H7 antibodies comprising a heavy chain selected from Hu1H7VHv1-5 and a light chain selected from Hu1H7VLv1-4 have been characterized.

[0120] Biacore full binding kinetic analysis of antibodies were carried out using Biacore. Detailed binding kinetic parameters (association rate, ka, dissociation rate, kd, and affinity constant, KD) were determined for murine 1H7 (Fig. 3A), chimeric 1H7 (Fig. 3B) and humanized 1H7 (Hu1H7VHv3-Hu1H7VLv3, Hu1H7VHv3-Hu1H7VLv1, Hu1H7VHv4-Hu1H7VLv1) antibodies (Fig. 3C). Binding kinetic parameters of humanized 1H7, in particular Hu1H7VHv3-Hu1H7VLv3, are comparable to those of murine 1H7.

Table 3. Binding kinetic parameters of murine 1H7, chimeric 1H7 and humanized 1H7 (Hu1H7VHv3-Hu1H7VLv3, Hu1H7VHv3-Hu1H7VLv1, Hu1H7VHv4-Hu1H7VLv1)

Antibody	**ka (1/Ms)**	**kd (1/s)**	**K_D (nM)**
Murine 1H7	1.0e6	9.6e-3	9.5
Chimeric 1H7	1.7e6	1.3e-2	7.4
Humanized 1H7 H3L3 (Hu1H7VHv3-Hu1H7VLv3)	1.3e6	1.1e-2	9.0

(continued)

Antibody	ka (1/Ms)	kd (1/s)	K_D (nM)
Humanized 1H7 H3L1 (Hu1H7VHv3-Hu1H7VLv1)	1.1e6	1.3e-2	12
Humanized 1H7 H4L1 (Hu1H7VHv4-Hu1H7VLv1)	9.7e5	3.3e-2	33.9
Humanized 1H7 H5L1 (Hu1H7VHv5-Hu1H7VLv1)	8.8e5	5.6e-2	64.0
Humanized 1H7 H4L4 (Hu1H7VHv4-Hu1H7VLv4)	N/A *	N/A *	N/A *

The binding kinetic parameters could not be determined for Hu1H7VHv4-Hu1H7VLv4 at concentrations comparable to those used in the analysis for other humanized 1H7 antibodies or murine/chimeric 1H7.

[0121] Binding kinetic of humanized 1H7 antibodies were also measured by bio-layer interferometry (BLI) using a ForteBio Octet QK instrument (ForteBio, Menlo Park, CA). Detailed binding kinetic parameters (association rate, apparent ka, dissociation rate, apparent kd, and affinity constant, apparent KD) were determined for chimeric 1H7 and various humanized 1H7 antibodies (Tables 6-7, Figs. 4A-C). Apparent ka, apparent kd and apparent K_D are binding kinetic parameters obtained using ForteBio assay formats. These parameters differ from ka, kd and K_D measured using Biocore assays due to, e.g., avidity effects associated with ForteBio assay formats.

Table 4. Binding kinetic parameters of chimeric 1H7 and humanized 1H7 (Hu1H7VHv1-Hu1H7VLv1, Hu1H7VHv1-Hu1H7VLv2, Hu1H7VHu1-Hu1H7VLv3, Hu1H7VHv1-Hu1H7VLv4, Hu1H7VHv2-Hu1H7VLv1, Hu1H7VHv2-Hu1H7VLv2)

Antibody	Apparent ka (1/Ms)	Apparent kd (1/s)	Apparent K_D (nM)
Chimeric 1H7	4.7e5	1.1e-6	2.3e-3
Humanized 1H7 H1L1 (Hu1H7VHv1-Hu1H7VLv1)	2.5e5	1.2e-3	4.8
Humanized 1H7 H1L2 (Hu1H7VHv1-Hu1H7VLv2)	1.6e6	9e-3	5.8
Humanized 1H7 H1L3 (Hu1H7VHv1-Hu1H7VLv3)	2.2e5	6.4e-4	2.9
Humanized 1H7 H1L4 (Hu1H7VHv1-Hu1H7VLv4)	1.8e6	5.8e-3	3.3
Humanized 1H7 H2L1 (Hu1H7VHv2-Hu1H7VLv1)	5.8e5	4.2e-6	7.2e-3
Humanized 1H7 H2L2 (Hu1H7VHv2-Hu1H7VLv2)	2.3e6	4.6e-3	2

Table 5. Binding kinetic parameters of chimeric 1H7 and humanized 1H7 (Hu1H7VHv4-Hu1H7VLv2, Hu1H7VHv4-Hu1H7VLv3, Hu1H7VHv4-Hu1H7VLv4, Hu1H7VHv5-Hu1H7VLv2, Hu1H7VHv5-Hu1H7VLv3, Hu1H7VHv5-Hu1H7VLv4)

Antibody	Apparent ka (1/Ms)	Apparent kd (1/s)	Apparent K_D (nM)
Chimeric 1H7	8.3e5	8.7e-4	1.0
Humanized 1H7 H4L2 (Hu17VHv4-Hu1H7VLv2)	1.2e6	2.1e-3	1.7
Humanized 1H7 H4L3 (Hu1H7VHv4-Hu1H7VLv3)	7.4e5	2.0e-3	2.7

(continued)

Antibody	Apparent ka (1/Ms)	Apparent kd (1/s)	Apparent K_D (nM)
Humanized 1H7 H4L4 (Hu1H7VHv4-Hu1H7VLv4)	5.2e5	2.3e-3	4.4
Humanized 1H7 H5L2 (Hu1H7VHv5-Hu1H7VLv2)	5.2e5	2.1e-3	4.0
Humanized 1H7 H5L3 (Hu1H7VHv5-Hu1H7VLv3)	7.6e5	2.0e-3	2.6
Humanized 1H7 H5L4 (Hu1H7VHv5-Hu1H7VLv4)	1.6e6	1.9e-3	1.2

[0122] Humanized 1H7 antibodies, in particular Hu1H7VHv3-Hu1H7VLv3 and Hu1H7VHv3-Hu1H7VLv1, exhibited staining patterns of various regions (e.g., striatum, pyramidal cell layer, cortex, substantia nigra) of transgenic or non-transgenic mouse brain similar to those of murine 1H7.

EXAMPLE II. Passive Immunization with α-synuclein antibodies

[0123] The goal of this experiment is to determine effectiveness of α-synuclein antibodies in *in vitro* and *in vivo* studies as well as behavioral assays. We used α-synuclein transgenic (Line 61), α-synuclein knockout and wildtype female mice, 3-4 months old at initiation and n=14/group. Antibodies tested included 9E4 (IgG1, epitope: amino acids 118-126 of alpha synuclein), 5C1 (IgG1, epitope: amino acids 118-126 of alpha synuclein, c-linker), 5D12, IgG2 (SN118-126), 1H7, IgG1 (SN 91-99) and an IgG1 control antibody 27-1. Mice received a dosage of 10 mg/kg over a 5 month period, for a total of 21 injections. In addition, the animals were injected with lentivirus (LV) expressing human α-synuclein (wt) by unilateral introduction of human α-synuclein (wt) into the hippocampus..

[0124] Readout antibodies include those from Chemicon (epitope: full-length alpha synuclein), Millipore (epitope: full-length alpha synuclein), and Neotope, ELADW 105 (epitope: amino acids 121-124 of full-length alpha synuclein).

[0125] **Endpoints:** Antibody titers were measured during the in life phase. Behavioral assays include Morris Water Maze test (MWW) and horizontal beam test. The round beam test is a test of motor balance, coordination and gait conducted using two beams of varying diameter. Beam A is the larger diameter (easier, considered the training beam) and Beam D is the smaller diameter (more difficult, considered the testing beam). Data is presented as "errors" (number of slips/10cm) and "speed" (time taken to travel 10cm/sec). Water maze performance was carried out at weeks 10 and termination. The following neuropathology measurements were taken: alpha synuclein aggregation, synaptophysin, and MAP2. The following biochemistry measurements were taken: alpha synuclein, PSD95, synaptophysin. Selected multilabeling and confocal labeling were carried out using synaptic, neuronal and glial markers.

[0126] The results showed that all antibodies, except 5D12, produced significant reduction in α-syn accumulation and preservation of synaptic and dendritic densities, as well as positive outcomes in MWM performance. The 9E4 antibody is effective in *in vitro* and *in vivo* studies as well as behavioral assays. Readouts indicate antibody may reduce neuritic/axonal alpha synuclein aggregates.

[0127] **Behavioral Results:** The 1H7 antibody (as well as 9E4 and 5C1 antibodies) improved water maze performance in α-synuclein transgenic mice, whereas 5D12 did not.. The 9E4 and 1H7 antibodies improved performance on the beam test as measured both by speed and errors, whereas the 5D12 and 5C1 antibodies did not (Fig. 6).

[0128] **Neuropathology Results:** The 9E4, 1H7 and 5C1 antibodies reduced ELADW-105 positive neuritic dystrophy, whereas the 5D12 antibody did not. In alpha synuclein transgenic mice, the 9E4 antibody reduced the area of neuropil by 43% in neocortex and by 40% in basal ganglia as compared to control. The 9E4 antibody also preserved synaptophysin and MAP2 in neocortex and basal ganglia.

DEPOSIT

[0129] The following monoclonal antibody-producing cell lines have been deposited under the provisions of the Budapest Treaty with the American Type Culture Collection (ATCC, P.O. Box 1549, Manassas, VA 20108) on the dates indicated:

Monoclonal antibody	Cell Line	Epitope/Specificity	Isotype	Date of Deposit	Accession No.
1H7	JH17.1H7.4.24.34	alpha-synuclein residues 91-99	IgG1	Feb. 26, 2007	PTA-8220

[0130] Unless otherwise apparent from the context, any step, feature, embodiment, or aspect can be used in combination with any other.

SEQUENCE LISTING

[0131]

SEQ ID NO:1 Natural human wildtype alpha-synuclein

MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVAT
VAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQ
EGILEDMPVDPDNEAYEMPSEEGYQDYEPEA

SEQ ID NO:2 Non-amyloid component (NAC) domain of alpha-synuclein as reported by Jensen et al.
EQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFV

SEQ ID NO:3 Non-amyloid component (NAC) domain of alpha-synuclein as reported by Ueda et al.
KEQVTNVGGAVVTGVTAVAQKTVEGAGS

SEQ ID NO:4 m1H7 antibody heavy chain variable nucleotide sequence (signal peptide underlined; CDRs shown in bold and underlined)

ATGGGATGGAGCTGGGTCTTTATCTTCCTCCTGTCAGGAACTGCAGGTGTCCATTG
CCAGGTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGACTTCAGTG
AAGATATCCTGCAAGGCTTCTGGCTACAGCTTCACA**AGCTACTATATACAC**TGG
GTGAAGCAGAGTCCTGGACAGGGACTTGAGTGGATTGGA**TGGATTTATCCTGGA**
AGTGGTAATACTAAGTACAGTGAGAAGTTCAAGGGCAAGGCCACACTGACTG
CAGACACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGA
CTCTGCAGTCTATTTCTGTGCAAGA**GATGGTTGCTACGGGTTTGCTTAC**TGGGG
CCAAGGGACTCTGGTCACTGTCTCT

SEQ ID NO:5 m1H7 antibody heavy chain variable aa sequence (signal peptide underlined; CDRs shown in bold and underlined)

MGWSWVFIFLLSGTAGVHCQVQLQQSGPELVKPGTSVKISCKASGYSFT**SYYIH**WVK
QSPGQGLEWIG**WIYPGSGNTKYSEKFKG**KATLTADTSSSTAYMQLSSLTSEDSAVY
FCAR**DGCYGFAY**WGQGTLVTVS

SEQ ID NO:6 m1H7 antibody light chain variable nucleotide sequence (signal peptide underlined; CDRs shown in bold and underlined)

ATGGAGACAGACACACTCCTGTTATGGGTGCTGCTGCTCTGGGTTCCAGGCTCCA
CTGGTGACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAG
AGGGCCACCATCTCCTGC**AAGGCCAGCCAAAGTGTTGATTATGATGGTGATAG**
TTATATGAACTGGTACCAACAGAAACCAGGACAGCCACCCAAATTCCTCATCTG
T**GCTGCATCCAATCTAGAATCT**GGGATCCCAGCCAGGTTTAGTGGCAGTGGGTC
TGGGACAGACTTCACCCTCAACATCCATCCTGTGGAGGAGGAGGATGCTGCAACC
TATTACTGT**CAGCAAAGTAATGAGGATCCATTCACG**TTCGGCTCGGGGACAAA
GTTGGAAATAAAA

SEQ ID NO:7 m1H7 antibody light chain variable aa sequence (signal peptide underlined; CDRs shown in bold and underlined)

METDTLLLWVLLLWVPGSTGDIVLTQSPASLAVSLGQRATISC**KASQSVDYDGDSY**
MNWYQQKPGQPPKFLIC**AASNLES**GIPARFSGSGSGTDFTLNIHPVEEEDAATYYC**Q**
QSNEDPFTFGSGTKLEIK

SEQ ID NO:8 mature m1H7 antibody heavy chain variable nucleotide sequence (CDRs shown in bold and underlined)

GTCCAGCTGCAGCAGTCTGGACCTGAGCTGGTGAAGCCTGGGACTTCAGTGAAGA
TATCCTGCAAGGCTTCTGGCTACAGCTTCACA**AGCTACTATATACAC**TGGGTGAA
GCAGAGTCCTGGACAGGGACTTGAGTGGATTGGA**TGGATTTATCCTGGAAGTG**
GTAATACTAAGTACAGTGAGAAGTTCAAGGGCAAGGCCACACTGACTGCAGAC
ACATCCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCTG
CAGTCTATTTCTGTGCAAGA**GATGGTTGCTACGGGTTTGCTTAC**TGGGGCCAAG
GGACTCTGGTCACTGTCTCT

SEQ ID NO:9 mature m1H7 antibody heavy chain variable aa sequence (CDRs shown in bold and underlined)

VQLQQSGPELVKPGTSVKISCKASGYSFT**SYYIH**WVKQSPGQGLEWIG**WIYPGSGNT**
KYSEKFKGKATLTADTSSSTAYMQLSSLTSEDSAVYFCAR**DGCYGFAY**WGQGTLV
TVS

SEQ ID NO:10 mature m1H7 antibody light chain variable nucleotide sequence (CDRs shown in bold and underlined)

GACATTGTGCTGACCCAATCTCCAGCTTCTTTGGCTGTGTCTCTAGGGCAGAGGG
CCACCATCTCCTGC**AAGGCCAGCCAAAGTGTTGATTATGATGGTGATAGTTAT**
ATGAACTGGTACCAACAGAAACCAGGACAGCCACCCAAATTCCTCATCTGT**GCT**
GCATCCAATCTAGAATCTGGGATCCCAGCCAGGTTTAGTGGCAGTGGGTCTGGG
ACAGACTTCACCCTCAACATCCATCCTGTGGAGGAGGAGGATGCTGCAACCTATT
ACTGT**CAGCAAAGTAATGAGGATCCATTCACG**TTCGGCTCGGGGACAAAGTTG
GAAATAAAA

SEQ ID NO:11 mature m1H7 antibody light chain variable aa sequence (CDRs shown in bold and underlined)

DIVLTQSPASLAVSLGQRATISC**KASQSVDYDGDSYMN**WYQQKPGQPPKFLIC**AASN**
LESGIPARFSGSGSGTDFTLNIHPVEEEDAATYYC**QQSNEDPFT**FGSGTKLEIK

SEQ ID NO:12 m1H7 antibody heavy chain CDR1 (Kabat definition)
SYYIH

SEQ ID NO:13 m1H7 antibody heavy chain CDR2 (Kabat definition)
WIYPGSGNTKYSEKFKG

SEQ ID NO:14 m1H7 antibody heavy chain CDR3 (Kabat definition)
DGCYGFAY

SEQ ID NO:15 m1H7 antibody light chain CDR1 (Kabat definition)
KASQSVDYDGDSYMN

SEQ ID NO:16 m1H7 antibody light chain CDR2 (Kabat definition)
AASNLES

SEQ ID NO:17 m1H7 antibody light chain CDR3 (Kabat definition)
OOSNEDPFT

SEQ ID NO:18 Hu1H7VHv1 (nucleotide seq)

CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGCTGAAGAAGCCCGGCGCCTCCGTG
AAGGTGTCCTGCAAGGCCTCCGGCTACTCCTTCACCTCCTACTACATCCACTGGGT
GAAGCAGGCCCCCGGCCAGGGCCTGGAGTGGATCGGCTGGATCTACCCCGGCTC
CGGCAACACCAAGTACTCCGAGAAGTTCAAGGGCCGCGCCACCCTGACCGCCGA
CACCTCCACCTCCACCGCCTACATGGAGCTGCGCTCCCTGCGCTCCGACGACACC
GCCGTGTACTTCTGCGCCCGCGACGGCTGCTACGGCTTCGCCTACTGGGGCCAGG
GCACCCTGGTGACCGTGTCCTCA

SEQ ID NO:19 Hu1H7VHv1 (amino acid seq) (CDRs shown in bold and underlined)

QVQLVQSGAELKKPGASVKVSCKASGYSFT**SYYIH**WVKQAPGQGLEWIG**WIYPGSG**
NTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGCYGFAY**WGQGT
LVTVSS

SEQ ID NO:20 Hu1H7VHv2 (nucleotide seq)

CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGCCTCCGTG
AAGGTGTCCTGCAAGGCCTCCGGCTACTCCTTCACCTCCTACTACATCCACTGGGT
GCGCCAGGCCCCCGGCCAGGGCCTGGAGTGGATCGGCTGGATCTACCCCGGCTCC
GGCAACACCAAGTACTCCGAGAAGTTCAAGGGCCGCGCCACCCTGACCGCCGAC
ACCTCCACCTCCACCGCCTACATGGAGCTGCGCTCCCTGCGCTCCGACGACACCG
CCGTGTACTTCTGCGCCCGCGACGGCTGCTACGGCTTCGCCTACTGGGGCCAGGG
CACCCTGGTGACCGTGTCCTCA

SEQ ID NO:21 Hu1H7VHv2 (amino acid seq) (CDRs shown in bold and underlined)

QVQLVQSGAEVKKPGASVKVSCKASGYSFT**SYYIH**WVRQAPGQGLEWIG**WIYPGS**
GNTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGCYGFAY**WGQG
TLVTVSS

SEQ ID NO:22 Hu1H7VHv3 (nucleotide seq)

CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGCCTCCGTG
AAGGTGTCCTGCAAGGCCTCCGGCTACACCTTCACCTCCTACTACATCCACTGGG
TGCGCCAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCTGGATCTACCCCGGCTC
CGGCAACACCAAGTACTCCGAGAAGTTCAAGGGCCGCGCCACCATGACCGCCGA
CACCTCCACCTCCACCGCCTACATGGAGCTGCGCTCCCTGCGCTCCGACGACACC
GCCGTGTACTACTGCGCCCGCGACGGCTGCTACGGCTTCGCCTACTGGGGCCAGG
GCACCCTGGTGACCGTGTCCTCA

SEQ ID NO:23 Hu1H7VHv3 (amino acid seq) (CDRs shown in bold and underlined)

QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYIH**WVRQAPGQGLEWMG**WIYPGS**
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCAR**DGCYGFAY**WGQ
GTLVTVSS

SEQ ID NO:24 Hu1H7VHv4 (nucleotide seq)

CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGCCTCCGTG
AAGGTGTCCTGCAAGGCCTCCGGCTACTCCTTCACCTCCTACTACATCCACTGGGT
GCGCCAGGCCCCCGGCCAGGGCCTGGAGTGGATCGGCTGGATCTACCCCGGCTCC
GGCAACACCAAGTACTCCGAGAAGTTCAAGGGCCGCGCCACCCTGACCGCCGAC
ACCTCCACCTCCACCGCCTACATGGAGCTGCGCTCCCTGCGCTCCGACGACACCG
CCGTGTACTTCTGCGCCCGCGACGGCTcCTACGGCTTCGCCTACTGGGGCCAGGGC
ACCCTGGTGACCGTGTCCTCA

SEQ ID NO:25 Hu1H7VHv4 (amino acid seq) (CDRs shown in bold and underlined)

QVQLVQSGAEVKKPGASVKVSCKASGYSFT**SYYIH**WVRQAPGQGLEWIG**WIYPGS**
GNTKYSEKFKGRATLTADTSTSTAYMELRSLRSDDTAVYFCAR**DGSYGFAY**WGQG
TLVTVSS

SEQ ID NO:26 Hu1H7VHv5 (nucleotide seq)

CAGGTGCAGCTGGTGCAGTCCGGCGCCGAGGTGAAGAAGCCCGGCGCCTCCGTG
AAGGTGTCCTGCAAGGCCTCCGGCTACACCTTCACCTCCTACTACATCCACTGGG
TGCGCCAGGCCCCCGGCCAGGGCCTGGAGTGGATGGGCTGGATCTACCCCGGCTC
CGGCAACACCAAGTACTCCGAGAAGTTCAAGGGCCGCGCCACCATGACCGCCGA
CACCTCCACCTCCACCGCCTACATGGAGCTGCGCTCCCTGCGCTCCGACGACACC
GCCGTGTACTACTGCGCCCGCGACGGCTcCTACGGCTTCGCCTACTGGGGCCAGG
GCACCCTGGTGACCGTGTCCTCA

SEQ ID NO:27 Hu1H7VHv5 (amino acid seq) (CDRs shown in bold and underlined)

QVQLVQSGAEVKKPGASVKVSCKASGYTFT**SYYIH**WVRQAPGQGLEWMG**WIYPGS**
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCAR**DGSYGFAY**WGQ
GTLVTVSS

SEQ ID NO:28 Hu1H7VH signal peptide (nucleotide seq)

ATGGAGTTCGGCCTGTCCTGGCTGTTCCTGGTGGCCATCCTGAAGGGCGTGCAGT
GC

SEQ ID NO:29 Hu1H7VH signal peptide (amino acid seq)
MEFGLSWLFLVAILKGVQC

SEQ ID NO:30 Hu1H7VH signal peptide (nucleotide seq)

ATGGACTGGACCTGGAGCATCCTTTTCTTGGTGGCAGCAGCAACAGGTGCCCACT
CC

SEQ ID NO:31 Hu1H7VH signal peptide (amino acid seq)
MDWTWSILFLVAAATGAHS

SEQ ID NO:32 Hu1H7VLv1 (nucleotide seq)

GACATCCAGCTGACCCAGTCCCCCTCCTCCCTGTCCGCCTCCGTGGGCGACCGCG
TGACCATCACCTGCAAGGCCTCCCAGTCCGTGGACTACGACGGCGACTCCTACAT
GAACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGTTCCTGATCTGCGCCGCC

TCCAACCTGGAGTCCGGCGTGCCCTCCCGCTTCTCCGGCTCCGGCTCCGGCACCG
ACTTCACCCTGACCATCTCCTCCCTGCAGCCCGAGGACGCCGCCACCTACTACTG
CCAGCAGTCCAACGAGGACCCCTTCACCTTCGGCCAGGGCACCAAGCTGGAGATC
AAG

SEQ ID NO:33 Hu1H7VLv1 (amino acid seq) (CDRs shown in bold and underlined)

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIC**AASN**
LESGVPSRFSGSGSGTDFTLTISSLQPEDAATYYC**QQSNEDPFT**FGQGTKLEIK

SEQ ID NO:34 Hu1H7VLv2 (nucleotide seq)

GACATCCAGCTGACCCAGTCCCCCTCCTCCCTGTCCGCCTCCGTGGGCGACCGCG
TGACCATCACCTGCAAGGCCTCCCAGTCCGTGGACTACGACGGCGACTCCTACAT
GAACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGTTCCTGATCTaCGCCGCC
TCCAACCTGGAGTCCGGCGTGCCCTCCCGCTTCTCCGGCTCCGGCTCCGGCACCG
ACTTCACCCTGACCATCTCCTCCCTGCAGCCCGAGGACGCCGCCACCTACTACTG
CCAGCAGTCCAACGAGGACCCCTTCACCTTCGGCCAGGGCACCAAGCTGGAGATC
AAG

SEQ ID NO:35 Hu1H7VLv2 (amino acid seq) (CDRs shown in bold and underlined)

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIY**AASN**
LESGVPSRFSGSGSGTDFTLTISSLQPEDAATYYC**QQSNEDPFT**FGQGTKLEIK

SEQ ID NO:36 Hu1H7VLv3 (nucleotide seq)

GACATCCAGCTGACCCAGTCCCCCTCCTCCCTGTCCGCCTCCGTGGGCGACCGCG
TGACCATCACCTGCAAGGCCTCCCAGTCCGTGGACTACGACGGCGACTCCTACAT
GAACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGTTCCTGATCTGCGCCGCC
TCCAACCTGGAGTCCGGCGTGCCCTCCCGCTTCTCCGGCTCCGGCTCCGGCACCG
ACTTCACCCTGACCATCTCCTCCCTGCAGCCCGAGGACTTCGCCACCTACTACTGC
CAGCAGTCCAACGAGGACCCCTTCACCTTCGGCCAGGGCACCAAGCTGGAGATC
AAG

SEQ ID NO:37 Hu1H7VLv3 (amino acid seq) (CDRs shown in bold and underlined)

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIC**AASN**
LESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQSNEDPFT**FGQGTKLEIK

SEQ ID NO:38 Hu1H7VLv4 (nucleotide seq)

GACATCCAGCTGACCCAGTCCCCCTCCTCCCTGTCCGCCTCCGTGGGCGACCGCG
TGACCATCACCTGCAAGGCCTCCCAGTCCGTGGACTACGACGGCGACTCCTACAT
GAACTGGTACCAGCAGAAGCCCGGCAAGGCCCCCAAGTTCCTGATCTaCGCCGCC
TCCAACCTGGAGTCCGGCGTGCCCTCCCGCTTCTCCGGCTCCGGCTCCGGCACCG
ACTTCACCCTGACCATCTCCTCCCTGCAGCCCGAGGACttCGCCACCTACTACTGCC
AGCAGTCCAACGAGGACCCCTTCACCTTCGGCCAGGGCACCAAGCTGGAGATCA
AG

SEQ ID NO:39 Hu1H7VLv4 (amino acid seq) (CDRs shown in bold and underlined)

DIQLTQSPSSLSASVGDRVTITC**KASQSVDYDGDSYMN**WYQQKPGKAPKFLIY**AASN**
LESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYC**QQSNEDPFT**FGQGTKLEIK

SEQ ID NO:40 Hu1H7VL signal peptide (nucleotide seq)

ATGGACATGCGCGTGCCCGCCCAGCTGCTGGGCCTGCTGATGCTGTGGGTGTCCG
GCTCCTCCGGC

SEQ ID NO:41 Hu1H7VL signal peptide (amino acid seq)
MDMRVPAQLLGLLMLWVSGSSG

SEQ ID NO:42 BAC02037 (GI-21670055) Human acceptor used for heavy chain Framework Amino acid seq

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSFGISWVRQAPGQGLEWMGWISPYNG
DTNYAQNLQGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDRGSMSDYWGQGT
LVTVSS

SEQ ID NO:43 AAY33358 GI-63102905) Human acceptor used for light chain Framework Amino acid seq

DIQLTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQKPGKAPKLLIYAASSLQSGV
PSRFSGSGSGTDFTLTISSLQPEDFATYYCLQDYNYPPTFGQGTKLEIK

SEQ ID NO:44 Hu1H7VH no backmutation or CDR mutation

QVQLVQSGAEVKKPGASVKVSCKASGYSFTSYYIHWVRQAPGQGLEWMGWIYPGS
GNTKYSEKFKGRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDGCYGFAYWGQG
TLVTVSS

SEQ ID NO:45 Hu1H7VL no backmutation or CDR mutation

DIQLTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPKLLIYAASN
LESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNEDPFTFGQGTKLEIK

SEQ ID NO:46 Hu1H7VH alternatives

QVQLVQSGAE-X_1-KKPGASVKVSCKASGY-X_2-FTSYYIHWV-X_3-QAPGQGLEW-X_4-
GWIYPGSGNTKYSEKFKGR-X_5-T-X_6-T-X_7-DTSTSTAYMELRSLRSDDTAVY-X_8-
CARDG-X_9-YGFAYWGQGTLVTVSS

wherein -X_1- is V or L; -X_2- is S or T; -X_3- is R or K; -X_4- is M or I; -X_5- is V or A; -X_6- is M or L; -X_7- is T or A; -X_8- is Y or F; -X_9- is C or S.

SEQ ID NO:47 Hu1H7VL alternatives

DIQLTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPK-Z_1-LI-Z_2-
AASNLESGVPSRFSGSGSGTDFTLTISSLQPED-Z_3-ATYYCQQSNEDPFTFGQGTKLEIK

wherein -Z_1- is L or F; -Z_2- is Y or C; -Z_3- is F or A.

SEQ ID NO:48 Hu1H7VH CDR3 alternatives
DG-X_9-YGFAY
wherein -X_9- is C or M or S or T, preferably C

SEQ ID NO:49 humanized 1H7 light chain constant region (with R) (common for v1-v4)

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:50 humanized 1H7 heavy chain constant region (common for v1-v5) IgG1

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNVKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:51 humanized 1H7 light chain constant region (without R) (common for v1-v4)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTE
QDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:52 humanized 1H7 heavy chain constant region (Glm3 allotype)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY
SLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLFP
PKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSV
LTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTC
LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH
EALHNHYTQKSLSLSPGK

SEQ ID NO:53 humanized 1H7 light chain version 3 (variable region + constant region with Arginine)

DIQLTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPKFLICAASN
LESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNEDPFTFGQGTKLEIKRTVAA
PSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK
DSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:54 humanized 1H7 light chain version 3 (variable region + constant region without Arginine)

DIQLTQSPSSLSASVGDRVTITCKASQSVDYDGDSYMNWYQQKPGKAPKFLICAASN
LESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSNEDPFTFGQGTKLEIKTVAAPS
VFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS
TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO:55 humanized 1H7 heavy chain version 3 (variable region + constant region)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIYPGS
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCARDGCYGFAYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH
TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC
PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV

HNVKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK
GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV
LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:56 humanized 1H7 heavy chain version 3 (variable region + constant region G1m3 allotype)

QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYYIHWVRQAPGQGLEWMGWIYPGS
GNTKYSEKFKGRATMTADTSTSTAYMELRSLRSDDTAVYYCARDGCYGFAYWGQG
TLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST
YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKN
QVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF
SCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:57 humanized 1H7 heavy chain constant region (IgG2)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPPVAG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKTKPREE
QFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPREPQVYTL
PPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPMLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

SEQ ID NO:58 humanized 1H7 heavy chain constant region (G1m1 allotype)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ
SSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPEL
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV
YTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

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Patentkrav

1. Antistof, der omfatter et variabelt område af den mature tungkæde med en aminosyresekvens, der er betegnet SEQ ID NO: 23, og et variabelt område af letkæden med en aminosyresekvens, der er betegnet SEQ ID NO: 33 eller 37, hvor antistoffet specifikt binder til humant alpha-synuclein.

2. Antistof ifølge krav 1, hvor det variable område af letkæden har en aminosyresekvens, der er betegnet SEQ ID NO: 37.

3. Antistof ifølge krav 1, hvor det variable område af letkæden har en aminosyresekvens, der er betegnet SEQ ID NO: 33.

4. Antistof ifølge et hvilket som helst af de foregående krav, hvor det variable område af den mature tungkæde er kondenseret til et konstant område af tungkæden og det konstante område af den mature letkæde er kondenseret til et konstant område af letkæden.

5. Antistof ifølge krav 4, hvor det konstante område af tungkæden har aminosyresekvensen, der er betegnet SEQ ID NO: 52, forudsat den C-terminale lysinrest kan udelades.

6. Antistof ifølge krav 4, hvor det konstante område af letkæden har aminosyresekvensen, der er betegnet SEQ ID NO: 49.

7. Antistof ifølge krav 4 eller 6, hvor det konstante område af tungkæden er af human IgG1-isotype.

8. Nukleinsyre, der koder for et variabelt område af den mature tungkæde og et variabelt område af den mature letkæde som defineret ifølge et hvilket som helst foregående krav.

9. Nukleinsyre ifølge krav 8 med en sekvens, der omfatter en hvilken som helst af SEQ ID NO: 22, 32 eller 36.

10. Værtscelle, der omfatter en vektor omfattende nukleinsyren ifølge krav 8 eller 9.

11. Farmaceutisk sammensætning, der omfatter et antistof som defineret ifølge et hvilket som helst af krav 1 til 7.

12. Antistof som defineret ifølge et hvilket som helst af krav 1 til 7 til anvendelse i behandling af en patient, der har eller har en risiko for at få en Lewy body-sygdom.

13. Antistoffet til anvendelse ifølge krav 12, hvor sygdommen er Parkinsons sygdom.

14. Fremgangsmåde til fremstilling af et antistof, hvilken fremgangsmåde omfatter dyrkning af celler transformeret med nukleinsyrer, der koder for antistoffets tung- og letkæder, således at cellerne sekreterer antistoffet; og oprensning af antistoffet fra celledyrkningsmediet; hvor antistoffet er defineret ifølge et hvilket som helst af krav 1 til 7.

15. Fremgangsmåde til fremstilling af en cellelinje, der producerer et antistof, hvilken fremgangsmåde omfatter indføring af en vektor, der koder for tung- og letkæder af et antistof og en egnet markør, i celler; opformering af cellerne under betingelser for udvælgelse af celler med øget kopital af vektoren; isolering af enkelte celler fra den udvalgte celle; og oprettelse af en bank med celler, der er klonet fra en enkelt celle, og som er udvalgt på baggrund af antistofudbyttet; hvor antistoffet er et antistof som defineret ifølge et hvilket som helst af krav 1 til 7.

16. Fremgangsmåde ifølge krav 15, der endvidere omfatter opformering af cellerne under selektive betingelser og screening for cellelinjer, der naturligt udtrykker og sekreterer mindst 100 mg/L/10^6 celler/24h af antistoffet ifølge et hvilket som helst af krav 1 til 7.

1H7 VH alignment

Kabat Numbering	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
m1H7VH	Q	V	Q	L	Q	Q	S	G	P	E	L	V	K	P	G	T	S	V	K	I
BAC02037	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V
Hu1H7VHv1	Q	V	Q	L	V	Q	S	G	A	E	L	K	K	P	G	A	S	V	K	V
Hu1H7VHv2	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V
Hu1H7VHv3	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V
Hu1H7VHv4	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V
Hu1H7VHv5	Q	V	Q	L	V	Q	S	G	A	E	V	K	K	P	G	A	S	V	K	V

Kabat Numbering	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40
m1H7VH	S	C	K	A	S	G	Y	S	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	K	Q	S
BAC02037	S	C	K	A	S	G	Y	T	F	T	***S***	***F***	***G***	***I***	***S***	W	V	R	Q	A
Hu1H7VHv1	S	C	K	A	S	G	Y	S	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	K	Q	A
Hu1H7VHv2	S	C	K	A	S	G	Y	S	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	R	Q	A
Hu1H7VHv3	S	C	K	A	S	G	Y	T	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	R	Q	A
Hu1H7VHv4	S	C	K	A	S	G	Y	S	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	R	Q	A
Hu1H7VHv5	S	C	K	A	S	G	Y	T	F	T	***S***	***Y***	***Y***	***I***	***H***	W	V	R	Q	A

Kabat Numbering	41	42	43	44	45	46	47	48	49	50	51	52	52A	53	54	55	56	57	58	59
m1H7VH	P	G	Q	G	L	E	W	I	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***
BAC02037	P	G	Q	G	L	E	W	M	G	***W***	***I***	***S***	***P***	***Y***	***N***	***G***	***D***	***T***	***N***	***Y***
Hu1H7VHv1	P	G	Q	G	L	E	W	I	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***
Hu1H7VHv2	P	G	Q	G	L	E	W	I	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***
Hu1H7VHv3	P	G	Q	G	L	E	W	M	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***
Hu1H7VHv4	P	G	Q	G	L	E	W	I	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***
Hu1H7VHv5	P	G	Q	G	L	E	W	M	G	***W***	***I***	***Y***	***P***	***G***	***S***	***G***	***N***	***T***	***K***	***Y***

FIG. 1A

1H7 VH alignment

Kabat Numbering	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79
m1H7VH	S	E	K	F	K	G	K	A	T	L	T	A	D	T	S	S	S	T	A	Y
BAC02037	A	Q	N	L	Q	G	R	V	T	M	T	T	D	T	S	T	S	T	A	Y
Hu1H7VHv1	S	E	K	F	K	G	R	A	T	L	T	A	D	T	S	T	S	T	A	Y
Hu1H7VHv2	S	E	K	F	K	G	R	A	T	L	T	A	D	T	S	T	S	T	A	Y
Hu1H7VHv3	S	E	K	F	K	G	R	A	T	M	T	A	D	T	S	T	S	T	A	Y
Hu1H7VHv4	S	E	K	F	K	G	R	A	T	L	T	A	D	T	S	T	S	T	A	Y
Hu1H7VHv5	S	E	K	F	K	G	R	A	T	M	T	A	D	T	S	T	S	T	A	Y

Kabat Numbering	80	81	82	82A	82B	82C	83	84	85	86	87	88	89	90	91	92	93	94	95	96
m1H7VH	M	Q	L	S	S	L	T	S	E	D	S	A	V	Y	F	C	A	R	D	G
BAC02037	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	Y	C	A	R	D	R
Hu1H7VHv1	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	F	C	A	R	D	G
Hu1H7VHv2	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	F	C	A	R	D	G
Hu1H7VHv3	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	Y	C	A	R	D	G
Hu1H7VHv4	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	F	C	A	R	D	G
Hu1H7VHv5	M	E	L	R	S	L	R	S	D	D	T	A	V	Y	Y	C	A	R	D	G

Kabat Numbering	97	98	99	100	101	102	103	104	105	106	107	108	109	110	111	112	113
m1H7VH	C	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	-
BAC02037	G	S	M	S	D	Y	W	G	Q	G	T	L	V	T	V	S	S
Hu1H7VHv1	C	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S
Hu1H7VHv2	C	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S
Hu1H7VHv3	C	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S
Hu1H7VHv4	S	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S
Hu1H7VHv5	S	Y	G	F	A	Y	W	G	Q	G	T	L	V	T	V	S	S

FIG. 1B

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1H7 VH alignment

Kabat Numbering	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
m1H7VL	D	I	V	L	T	Q	S	P	A	S	L	A	V	S	L	G	Q	R	A	T
AAY33358	D	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T
Hu1H7VLv1	D	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T
Hu1H7VLv2	D	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T
Hu1H7VLv3	D	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T
Hu1H7VLv4	D	I	Q	L	T	Q	S	P	S	S	L	S	A	S	V	G	D	R	V	T

Kabat Numbering	21	22	23	24	25	26	27	27A	27B	27C	27D	28	29	30	31	32	33	34	35	36
m1H7VL	I	S	C	K	A	S	Q	S	V	D	Y	D	G	D	S	Y	M	N	W	Y
AAY33358	I	T	C	R	A	S	Q	-	-	-	-	G	I	R	N	D	L	G	W	Y
Hu1H7VLv1	I	T	C	K	A	S	Q	S	V	D	Y	D	G	D	S	Y	M	N	W	Y
Hu1H7VLv2	I	T	C	K	A	S	Q	S	V	D	Y	D	G	D	S	Y	M	N	W	Y
Hu1H7VLv3	I	T	C	K	A	S	Q	S	V	D	Y	D	G	D	S	Y	M	N	W	Y
Hu1H7VLv4	I	T	C	K	A	S	Q	S	V	D	Y	D	G	D	S	Y	M	N	W	Y

Kabat Numbering	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56
m1H7VL	Q	Q	K	P	G	Q	P	P	K	F	L	I	C	A	A	S	N	L	E	S
AAY33358	Q	Q	K	P	G	K	A	P	K	L	L	I	Y	A	A	S	S	L	Q	S
Hu1H7VLv1	Q	Q	K	P	G	K	A	P	K	F	L	I	C	A	A	S	N	L	E	S
Hu1H7VLv2	Q	Q	K	P	G	K	A	P	K	F	L	I	Y	A	A	S	N	L	E	S
Hu1H7VLv3	Q	Q	K	P	G	K	A	P	K	F	L	I	C	A	A	S	N	L	E	S
Hu1H7VLv4	Q	Q	K	P	G	K	A	P	K	F	L	I	Y	A	A	S	N	L	E	S

FIG. 2A

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1H7 VH alignment

Kabat Numbering	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76
m1H7VL	G	I	P	A	R	F	S	G	S	G	S	G	T	D	F	T	L	N	I	H
AAY33358	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S
Hu1H7VLv1	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S
Hu1H7VLv2	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S
Hu1H7VLv3	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S
Hu1H7VLv4	G	V	P	S	R	F	S	G	S	G	S	G	T	D	F	T	L	T	I	S

Kabat Numbering	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96
m1H7VL	P	V	E	E	E	D	A	A	T	Y	Y	C	Q	Q	S	N	E	D	P	F
AAY33358	S	L	Q	P	E	D	F	A	T	Y	Y	C	L	Q	D	Y	N	Y	P	P
Hu1H7VLv1	S	L	Q	P	E	D	A	A	T	Y	Y	C	Q	Q	S	N	E	D	P	F
Hu1H7VLv2	S	L	Q	P	E	D	A	A	T	Y	Y	C	Q	Q	S	N	E	D	P	F
Hu1H7VLv3	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	S	N	E	D	P	F
Hu1H7VLv4	S	L	Q	P	E	D	F	A	T	Y	Y	C	Q	Q	S	N	E	D	P	F

Kabat Numbering	97	98	99	100	101	102	103	104	105	106	107
m1H7VL	T	F	G	S	G	T	K	L	E	I	K
AAY33358	T	F	G	Q	G	T	K	L	E	I	K
Hu1H7VLv1	T	F	G	Q	G	T	K	L	E	I	K
Hu1H7VLv2	T	F	G	Q	G	T	K	L	E	I	K
Hu1H7VLv3	T	F	G	Q	G	T	K	L	E	I	K
Hu1H7VLv4	T	F	G	Q	G	T	K	L	E	I	K

FIG. 2B

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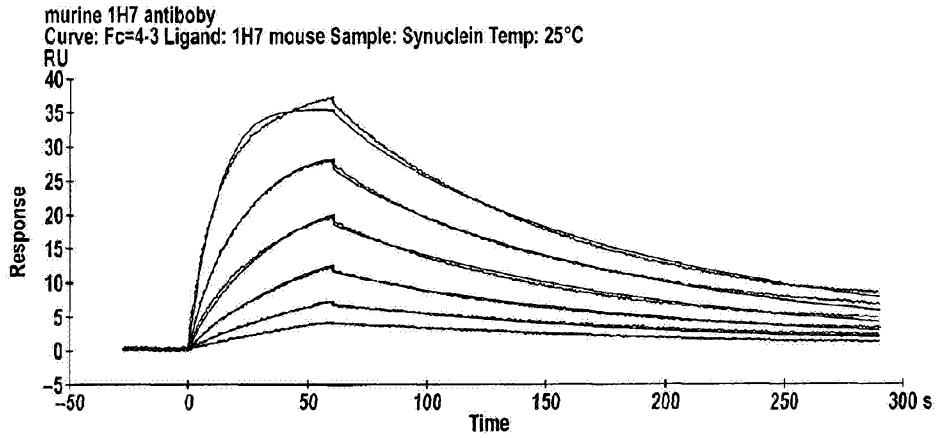


FIG. 3A

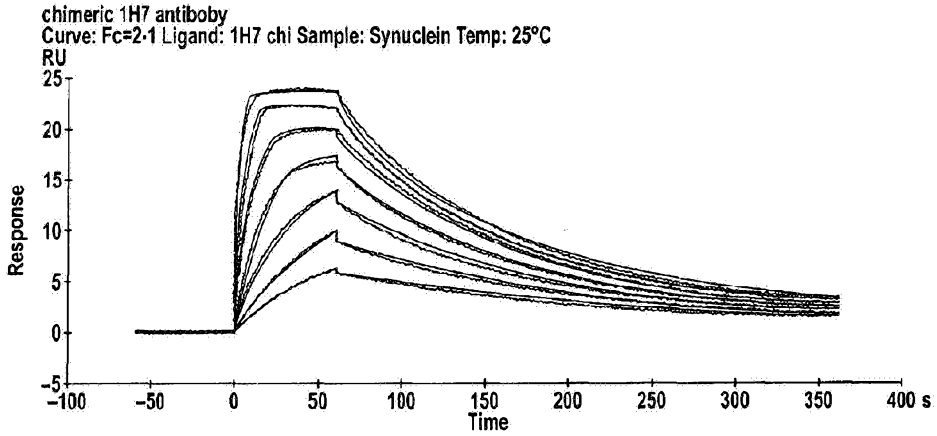


FIG. 3B

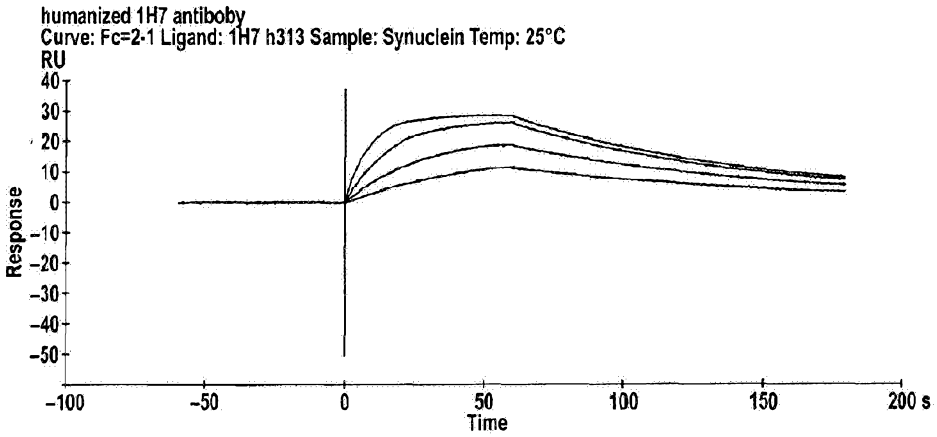


FIG. 3B

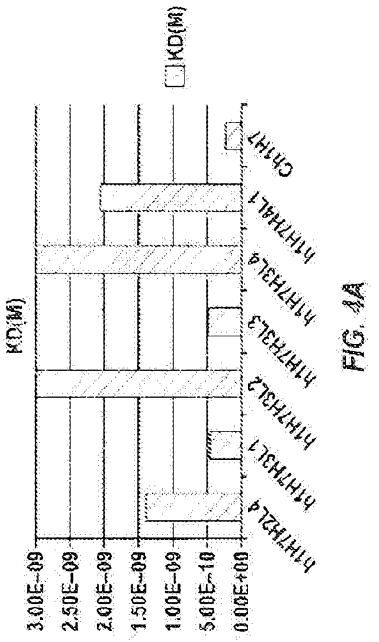


FIG. 4A

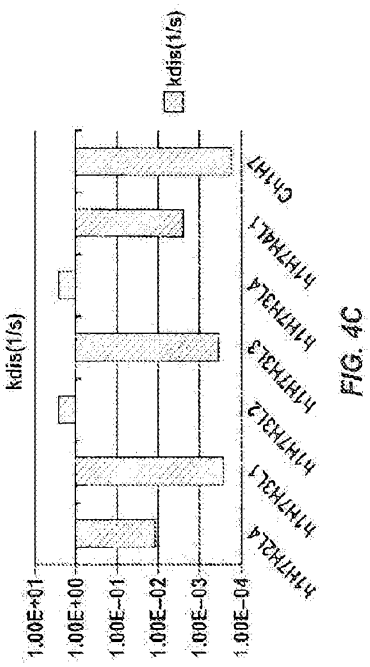


FIG. 4C

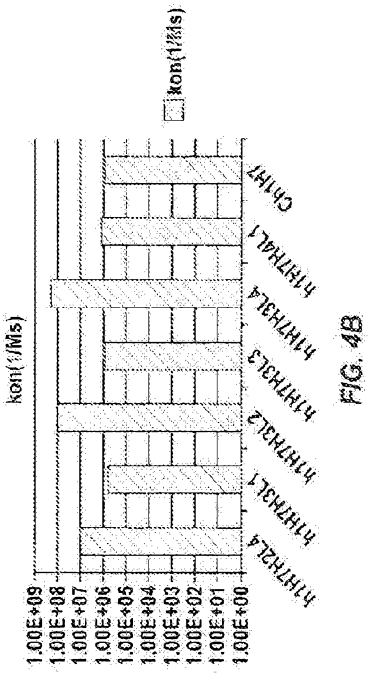
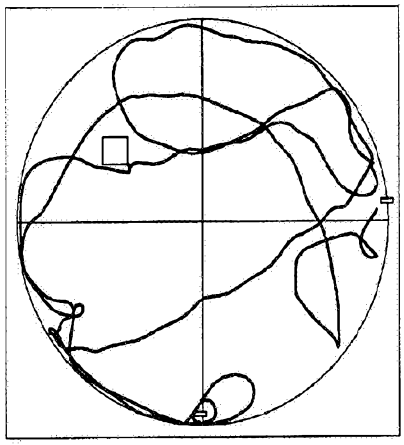


FIG. 4B



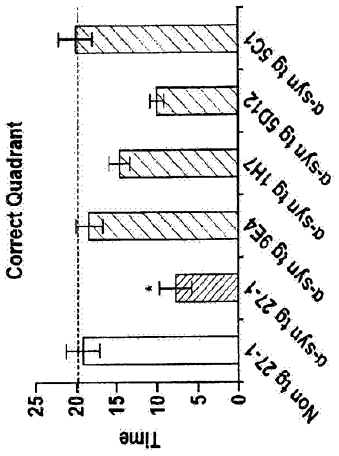

Correct Quadrant
Time
25
20
15
10
5
0
Non tg 27-1
α-syn tg 27-1
α-syn tg 9E4
α-syn tg 1H7
α-syn tg 5D12
α-syn tg 5C1

FIG. 5

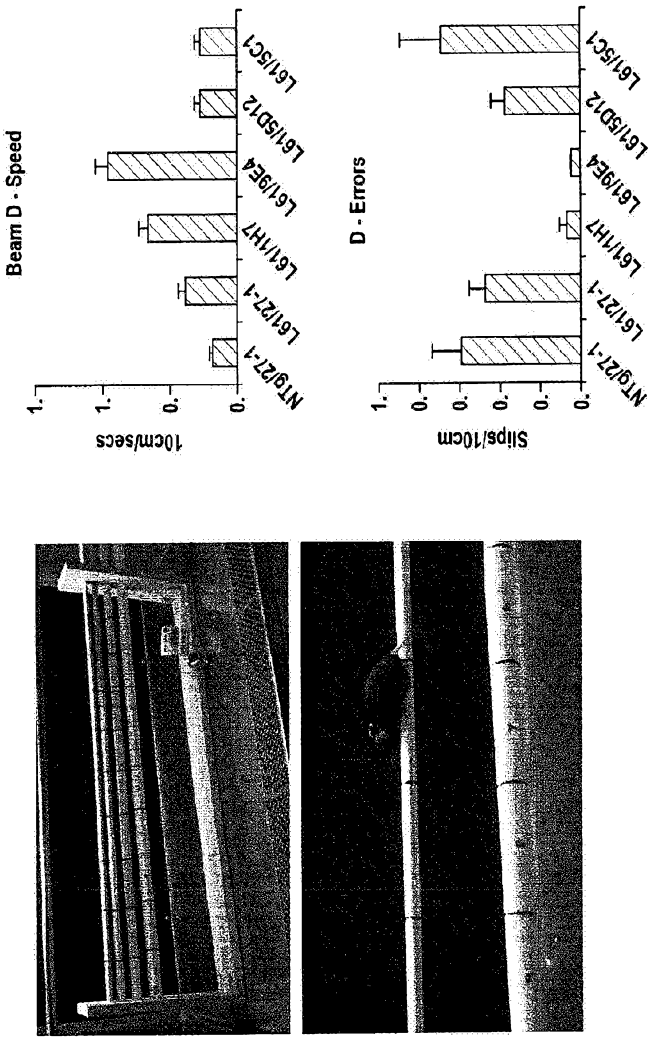

Beam D - Speed
10cm/secs
1.
1.
0.
0.
NTg/Z-1
L61/Z-1
L61/1H7
L61/9E4
L61/5D12
L61/5C1
D - Errors
Slips/10cm
1.
0.
0.
0.
0.
0.
NTg/Z-1
L61/Z-1
L61/1H7
L61/9E4
L61/5D12
L61/5C1

FIG. 6