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(54) Title: USE OF MIMOTOPES OF ALPHA-SYNUCLEIN EPITOPES FOR TREATING LEWY BODY DISEASES

(57) Abstract: The present invention relates to peptides or polypeptides for producing medicaments for preventing and/or treating synucleinopathies.

USE OF MIMOTOPES OF ALPHA-SYNUCLEIN EPITOPES FOR TREATING LEWY BODY DISEASES

The present invention relates to a medicament to be used to prevent and/or treat synucleinopathies.

Synucleinopathies are a diverse group of neurodegenerative disorders that share a common pathologic characteristic: in neuropathologic examinations characteristic lesions can be detected containing abnormal aggregates of alpha-synuclein (alpha-syn, a-syn) protein in selected populations of neurons and glia cells.

Alpha-syn (initially identified as PARK1 and PARK4) is a 140 amino acid protein widely expressed in the neocortex, hippocampus, dentate gyrus, olfactory bulb, striatum, thalamus and cerebellum. Alpha-Syn is also highly expressed in hematopoietic cells including B-, T-, and NK cells as well as monocytes and platelets. The exact role in these cells is not known but it has been implicated in the differentiation of megakaryocytes (platelet precursors).

The most common synucleinopathies include but are not limited to Lewy body disorders (LBDs) like Parkinson´s disease (PD), Parkinson`s disease with dementia (PDD) and dementia with Lewy bodies (DLB), as well as Multiple System Atrophy (MSA) or Neurodegeneration with Brain Iron Accumulation type I (NBIA Type I). The current treatment options for these diseases include symptomatic medications such as L-dopa, anticholinergic drugs as well as inhibitors of monoamine oxidase. However, all treatment opportunities currently present only lead to symptomatic alleviation but do not induce a long lasting, disease modifying effect in patients.

Lewy body disorders (LBD) are progressive neurodegenerative disorders characterized by tremor, rigidity, bradykinesia and by loss of dopaminergic neurons in the brain. In the case of DLB and PDD signs also include cognitive impairment. Up to 2% of the population above 60 years of age in western countries develop the typical signs of PD/LBD. Currently only symptomatic treatment is available. Unfortunately, these therapies only provide temporary relief from early symptoms and do not halt disease progression. The pathogenesis of PD/LBD is still incompletely understood, but it appears that genetic susceptibility and environmental factors are involved in the development of the disease. Despite all genetic advances, PD/LBD is primarily a sporadic disorder with no known cause (also called idiopathic

PD/LBD).

Patients suffering from this disease develop characteristic ubiquitinated intracellular inclusions called Lewy bodies (LBs) in the cortical and subcortical areas of the brain. Especially regions with high content of dopaminergic neurons or neuronal projections show this typical pathologic feature. Recently, several studies could show that the synaptic protein alpha-syn plays a central role in LBD pathogenesis. In LBD, alpha-syn accumulates in LBs throughout affected brain areas. Additionally, it could be demonstrated that single point mutations as well as duplications or multiplications in the alpha-syn gene are associated with rare familial forms of parkinsonism. Importantly, based on results from overexpression studies in transgenic (tg) mice as well as in Drosophila melanogaster its key role in the pathogenesis of PD/LBD is underscored as these animal models mimic several characteristics of PD.

Another very important synucleinopathy is Multiple System Atrophy (MSA). MSA is a sporadic neurodegenerative disorder that is characterised by symptoms of L-DOPA-resistant parkinsonism, cerebellar ataxia, and dysautonomia. Patients suffer from multisystem neuronal loss affecting various brain areas including striatum, substantia nigra, cerebellum, pons, as well as the inferior olives and the spinal cord. MSA is characterized by alpha- syn-positive glial cytoplasmic (GCI) and rare neuronal inclusions throughout the central nervous system. These inclusions are associated with striatonigral degeneration, olivopontocerebellar atrophy, and involvement of autonomic nuclei in medulla and spinal cord. The importance of GCIs for the pathogenesis of MSA is generally acknowledged and underscored by recent analysis of transgenic mouse models analysing the effect of alpha-syn overexpression in oligodendroglia. In tg mice overexpressing human alpha-syn both GCI-like aggregates and biochemical markers of MSA were observed.

Although the exact mechanisms by which accumulation of alpha- syn leads to the typical features of neurodegeneration in synucleopathies are not fully understood, recent studies imply that abnormal formation and accumulation of alpha-syn is involved in the degenerative processes underlying synucleinopathy. Recently, different forms of alpha-syn have been identified in LBs. Beside the full length form of the protein, different forms

of modified alpha-syn have been identified including phosphorylated, nitrated, and mono-, di-, or tri-ubiquitinated alpha-syn. In addition, C-terminally truncated forms of the protein, like alpha-syn 1-119, alpha-syn 1-122 and alpha-syn 1-123, have been detected in brain tissue from both transgenic mice and PD cases. It is currently believed that up to 15% of the alpha-syn detected in LBs and lewy neurites is truncated. Previous in vitro studies using truncated alpha-syn could demonstrate that alpha-syn lacking the C-terminal 20-30 amino acids was showing an increased tendency to aggregate and to form filaments found in Lewy-neurites and LBs. These truncated versions could thus act in a similar way as truncated and modified forms of amyloid beta (Aß)in Alzheimer´s disease (AD). These truncated and modified forms of Aß are thought to act as seed molecules for plaque deposition and show a higher aggregation propensity as well as high neurotoxicity and synaptotoxicity in vivo and in vitro.

Thus full length alpha-syn as well as truncated and/or modified forms of alpha-syn, which are showing potential seeding effects, are then believed to accumulate leading to oligomer-formation. Based on recent studies it is believed that such oligomer-formation for example in the synaptic terminals and axons plays an important role for PD/LBD development and could thus be enhanced by the presence of truncated forms of alpha-syn. Hence, reduction of alpha-syn deposition and oligomerisation should be beneficial in the treatment of synucleopathies, especially of idiopathic LBD/PD and MSA and could present the first strategy for treatment of these neurodegenerative diseases in addition to the mere alleviation of symptoms resulting from current treatment strategies like L-DOPA application.

In Iwatsubo T. (Neuropathology 27 (5)(2007): 474-478) the correlation of alpha-synuclein depositions as well as its phosphorylation with a pathogenesis of alpha-synucleopathies is examined. The author of this publication found that serine 129 of alpha-synuclein deposited in synucleopathy lesions is extensively phosphorylated. US 2007/213253 relates to mutant human alpha-synuclein as well as peptides derived therefrom which may be used for inhibiting the aggregation of the wild-type human alpha-synuclein. In the WO 2004/041067 means and methods for preventing or treating diseases associated with alpha-synuclein aggregation are disclosed which comprise the use of alpha-

synuclein fragments. In the US 2003/166558 peptides are described which can be used to induce immune response to protein deposits. US 2005/198694 relates to alpha-synuclein fragments comprising at least 100 amino acids and having a C-terminal deletion of 1 to 23 amino acids.

Liang et al. (J. Neurochem. 99(2006): 470-482) studied the regulation of alpha-synuclein in rats. They observed that in alcohol preferring rats the expression rate of alpha-synuclein is increased compared to alcohol-non preferring rats.

In Hamilton BA (Genomics 83(2004): 739-742) the distribution of alpha-synuclein 53Thr and 53Ala in primates is examined.

In US 2005/0037013 immunogenic alpha-synuclein fragments are disclosed which are able to induce an immune response against a specific epitope within residues 70-140 of alpha-synuclein.

WO 2006/045037 relates to C-terminal truncated alpha-synuclein molecules which can be used to screen for agents which have a pharmacological activity useful for treating a Lewy Body Disease.

Although experimental therapies utilizing neurotrophic factors and grafting of dopaminergic cells have yielded promising results, alternative approaches designed to reduce the neuronal accumulation of alpha-syn are required. There is compelling evidence accumulating that alpha-syn aggregates might be targeted by immunotherapy. Indeed, recently a potential for the treatment of synucleopathies has been shown. Tg mice overexpressing human alpha-syn were vaccinated with human alpha-syn protein. In mice that produced high relative affinity antibodies upon vaccination, there was decreased accumulation of aggregated alpha-syn in neuronal cell bodies and synapses which was associated with reduced neurodegeneration. Furthermore, antibodies produced by immunized animals also detected abnormal aggregated forms of alpha-syn associated with the neuronal membrane and promoted the degradation of these aggregates, probably via lysosomal pathways. Similar effects were observed using passive immunotherapy with an exogenously applied alpha-syn-specific antibody. These results suggest that vaccination is effective in reducing neuronal accumulation of alpha-syn aggregates and that further development of this approach might elicit beneficial effects in the treatment of LBD and synucleinopathies.

It is an object of the present invention to provide a me-

dicament to prevent and treat synucleinopathies on the basis of a vaccine.

The present invention relates to the provision of at least one peptide or polypeptide comprising the amino acid sequence

$(X_1)_nX_2X_3X_4X_5GX_6P(X_7)_m$ (Formula I),

wherein

X_1 is any amino acid residue,

X_2 is an amino acid residue selected from the group consisting of lysine (K), arginine (R), alanine (A) and histidine (H),

X_3 is an amino acid residue selected from the group consisting of asparagine (N), glutamine (Q), serine (S), glycine (G) and alanine (A), preferably asparagine (N), serine (S), glycine (G) and alanine (A),

X_4 is an amino acid residue selected from the group consisting of glutamic acid (E), aspartic acid (D) and alanine (A),

X_5 is an amino acid residue selected from the group consisting of glutamic acid (E) and aspartic acid (D),

X_6 is an amino acid residue selected from the group consisting of alanine (A) and tyrosine (Y),

X_7 is any amino acid residue,

n and m, independently, are 0 or an integer of more than 0,

and wherein the amino acid sequence according to Formula I is not identical with, or does not comprise the 7-mer polypeptide fragment of alpha-synuclein having the amino acid sequence KNEEGAP,

said at least one peptide or polypeptide having a binding capacity to an antibody which is specific for an epitope of alpha-synuclein comprising the amino acid sequence KNEEGAP,

for use in preventing and/or treating synucleinopathies.

These peptides or polypeptides according to the present invention can be provided in compositions suitable for the intended use for preventing and/or treating synucleinopathies, especially in pharmaceutical compositions, preferably combined with a pharmaceutically acceptable carrier. Such pharmaceutical compositions can be administered to a patient in need thereof in an effective amount to achieve the preventive and/or therapeutic effect.

The peptides and polypeptide according to the present invention are able to induce the *in vivo* formation of antibodies di-

rected (binding) to alpha-synuclein and fragments thereof, in particular to fragments of alpha-synuclein comprising the amino acid sequence KNEEGAP. Antibodies directed (binding) to said peptides and polypeptides, however, show no or substantially no immune reactivity to beta-synuclein (beta-syn, b-syn). Therefore, unlike the original alpha-synuclein or fragment(s) thereof, the peptides and polypeptides according to the present invention provide a specificity towards the disease related agent and avoid cross reactivity with disease unrelated synucleins. This strongly suggests significant superiority regarding efficacy and safety, the latter in particular because of the neuroprotective characteristics that have been described for beta-synuclein (Hashimoto M. et al., J Biol Chem. 2004 May 28;279(22):23622-9. Hashimoto M, Neuron. 2001 Oct 25;32(2):213-23).

The alpha-synuclein specific antibodies induced by the administration of the compounds of the present invention might not only bind to monomeric forms of alpha-synuclein but also to multimeric forms. This allows to reduce the amount of oligomers of alpha-synuclein in the body of an individual to be treated. The reduction of alpha-synuclein is particularly beneficial in the treatment of synucleopathies.

The amino acid sequence $(X_1)_nX_2X_3X_4X_5GX_6P(X_7)_m$ is considered to be a mimotope of the epitope of alpha-synuclein comprising the amino acid sequence KNEEGAP. According to the present invention the term "mimotope" refers to a molecule which has a conformation that has a topology equivalent to the epitope of which it is a mimic. The mimotope binds to the same antigen-binding region of an antibody which binds immunospecifically to a desired antigen. The mimotope will elicit an immunological response in a host that is reactive to the antigen to which it is a mimic. The mimotope may also act as a competitor for the epitope of which it is a mimic in in vitro inhibition assays (e.g. ELISA inhibition assays) which involve the epitope and an antibody binding to said epitope. However, a mimotope of the present invention may not necessarily prevent or compete with the binding of the epitope of which it is a mimic in an in vitro inhibition assay although it is capable to induce a specific immune response when administered to a mammal.

As used herein, the term "epitope" refers to an immunogenic

region of an antigen which is recognized by a particular antibody molecule. In general, an antigen will possess one or more epitopes, each capable of binding an antibody that recognizes the particular epitope.

The mimotopes of the present invention can be synthetically produced by chemical synthesis methods which are well known in the art, either as an isolated peptide or as a part of another peptide or polypeptide. Alternatively, the peptide mimotope can be produced in a microorganism which produces the peptide mimotope which is then isolated and if desired, further purified. The peptide mimotope can be produced in microorganisms such as bacteria, yeast or fungi, in eukaryote cells such as a mammalian or an insect cell, or in a recombinant virus vector such as adenovirus, poxvirus, herpesvirus, Simliki forest virus, baculovirus, bacteriophage, sindbis virus or sendai virus. Suitable bacteria for producing the peptide mimotope include E.coli, B.subtilis or any other bacterium that is capable of expressing peptides such as the peptide mimotope. Suitable yeast types for expressing the peptide mimotope include Saccharomyces cerevisiae, Schizosaccharomyces pombe, Candida, Pichia pastoris or any other yeast capable of expressing peptides. Corresponding methods are well known in the art. Also methods for isolating and purifying recombinantly produced peptides are well known in the art and include e.g. gel filtration, affinity chromatography, ion exchange chromatography etc.

To facilitate isolation of the peptide mimotope, a fusion polypeptide may be made wherein the peptide mimotope is translationally fused (covalently linked) to a heterologous polypeptide which enables isolation by affinity chromatography. Typical heterologous polypeptides are His-Tag (e.g. His_6; 6 histidine residues), GST-Tag (Glutathione-S-transferase) etc.. The fusion polypeptide facilitates not only the purification of the mimotopes but can also prevent the mimotope polypeptide from being degraded during purification. If it is desired to remove the heterologous polypeptide after purification the fusion polypeptide may comprise a cleavage site at the junction between the peptide mimotope and the heterologous polypeptide. The cleavage site consists of an amino acid sequence that is cleaved with an enzyme specific for the amino acid sequence at the site (e.g. proteases).

According to a preferred embodiment of the present invention, X_2 is an amino acid residue selected from the group consisting of lysine (K) and arginine (R) and/or X_6 is alanine (A).

According to a particularly preferred embodiment of the present invention, the peptide or polypeptide comprises an amino acid sequence selected from the group consisting of $(X_1)_nKNDEGAP(X_7)_m$, $(X_1)_nANEEGAP(X_7)_m$, $(X_1)_nKAEEGAP(X_7)_m$, $(X_1)_nKNAEGAP(X_7)_m$, $(X_1)_nRNEEGAP(X_7)_m$, $(X_1)_nHNEEGAP(X_7)_m$, $(X_1)_nKNEDGAP(X_7)_m$, $(X_1)_nKQEEGAP(X_7)_m$, $(X_1)_nKSEEGAP(X_7)_m$, $(X_1)_nKNDDGAP(X_7)_m$, $(X_1)_nRNDEGAP(X_7)_m$, $(X_1)_nRNEDGAP(X_7)_m$, $(X_1)_nRQEEGAP(X_7)_m$, $(X_1)_nRSEEGAP(X_7)_m$, $(X_1)_nANDEGAP(X_7)_m$, $(X_1)_nANEDGAP(X_7)_m$, $(X_1)_nHSEEGAP(X_7)_m$, $(X_1)_nASEEGAP(X_7)_m$, $(X_1)_nHNEDGAP(X_7)_m$, $(X_1)_nHNDEGAP(X_7)_m$, $(X_1)_nRNAEGAP(X_7)_m$, $(X_1)_nHNAEGAP(X_7)_m$, $(X_1)_nKSAEGAP(X_7)_m$, $(X_1)_nKSDEGAP(X_7)_m$, $(X_1)_nKSEDGAP(X_7)_m$, $(X_1)_nRQDEGAP(X_7)_m$, $(X_1)_nRQEDGAP(X_7)_m$, $(X_1)_nHSAEGAP(X_7)_m$, $(X_1)_nRSAEGAP(X_7)_m$, $(X_1)_nRSDEGAP(X_7)_m$, $(X_1)_nRSEDGAP(X_7)_m$, $(X_1)_nHSDEGAP(X_7)_m$, $(X_1)_nHSEDGAP(X_7)_m$, $(X_1)_nRQDDGAP(X_7)_m$, preferably $(X_1)_nKNDEGAP(X_2)_m$, $(X_1)_nRNEEGAP(X_2)_m$, $(X_1)_nRNDEGAP(X_2)_m$, $(X_1)_nKNAEGAP(X_2)_m$, $(X_1)_nKSDEGAP(X_2)_m$, $(X_1)_nRNAEGAP(X_2)_m$ or $(X_1)_nRSEEGAP(X_2)_m$.

It turned out that not only peptides and polypeptides according to formula I can be used in the treatment and prevention of synucleinopathies, but also other peptides and polypeptides. Therefore, another aspect of the present invention relates to at least one peptide or polypeptide comprising an amino acid sequence selected from the group consisting of $(X_1)_nKNEAGAP(X_7)_m$, $(X_1)_nKNEEAAP(X_7)_m$, $(X_1)_nKNEEGAA(X_7)_m$, $(X_1)_nKPSFKNE(X_7)_m$, $(X_1)_nQPSFAME(X_7)_m$, $(X_1)_nSPSFKQE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, $(X_1)_nDPSFALE(X_7)_m$, $(X_1)_nLPSFRLE(X_7)_m$, $(X_1)_nEPNSRMD(X_7)_m$, $(X_1)_nQPSSKLD(X_7)_m$, $(X_1)_nHIHQSKFFDAPP(X_7)_m$, $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nQPAFAME(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nQPASKLD(X_7)_m$, $(X_1)_nQPSFAMA(X_7)_m$, $(X_1)_nTPSWKGA(X_7)_m$, $(X_1)_nQPSSKLA(X_7)_m$, $(X_1)_nAPSWKGE(X_7)_m$, $(X_1)_nTPSAKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKAE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, especially an amino acid sequence selected from the group consisting of $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$,

wherein

X_1 is any amino acid residue,

X_7 is any amino acid residue,

n and m, independently, are 0 or an integer of more than 0,

said at least one peptide or polypeptide having a binding capacity to an antibody which is specific for an epitope of alpha-synuclein comprising the amino acid sequence KNEEGAP,

for use in preventing and/or treating synucleinopathies, especially for the production of a medicament therefor.

The peptides and polypeptides of the present invention may also be modified at or nearby their N- and/or C-termini so that at said positions a cysteine residue is bound thereto. In a preferred embodiment terminally positioned (located at the N- and C-termini of the peptide) cysteine residues are used to cross-link said molecules with carrier molecules such as KLH or to cyclize the peptides through a disulfide bond. Therefore, n and/or m are preferably 1 and X_1 and/or X_7 are preferably cysteine (C).

The mimotopes of the present invention may also be used in various assays and kits, in particular in immunological assays and kits. Therefore, it is particularly preferred that the peptides and polypeptides of the present invention may be part of another peptide or polypeptide, particularly an enzyme which is used as a reporter in immunological assays. Such reporter enzymes include e.g. alkaline phosphatase or horseradish peroxidase.

The alpha-synuclein mimotopes according to the present invention preferably are antigenic polypeptides which in their amino acid sequence vary from the amino acid sequence of alpha-synuclein or of fragments of alpha-synuclein. In this respect, the inventive mimotopes may not only comprise amino acid substitutions of one or more naturally occurring amino acid residues but also of one or more non-natural amino acids (i.e. not from the 20 "classical" amino acids) or they may be completely assembled of such non-natural amino acids. Moreover, the inventive antigens which induce anti-alpha-synuclein antibodies may be assembled of D- or L- amino acids or of combinations of DL- amino acids and, optionally, they may have been changed by further modifications, ring closures or derivatizations. Suitable antialpha-synuclein-antibody-inducing antigens may be provided from commercially available peptide libraries. Preferably, these peptides are at least 7 amino acids, and preferred lengths may be up to 16, preferably up to 14 or 20 amino acids residues

(e.g. 7 or 8 to 20, 7 or 8 to 16 etc.). Thus, the peptide or polypeptide of the present invention comprises 7 to 30, preferably 7 to 20, more preferably 7 to 16, most preferably 8, amino acid residues. According to the invention, however, also longer peptides may very well be employed as anti-alpha-synuclein-antibody-inducing antigens. Furthermore the mimotopes of the present invention may also be part of a polypeptide and consequently comprising at their N- and/or C-terminus at least one further amino acid residue.

For preparing alpha-synuclein mimotopes (i.e. anti-alpha-synuclein-antibody-inducing antigens), of course also phage libraries, peptide libraries are suitable, for instance produced by means of combinatorial chemistry or obtained by means of high throughput screening techniques for the most varying structures (Display: A Laboratory Manual by Carlos F. Barbas (Editor), et al.; Willats WG Phage display: practicalities and prospects. Plant Mol. Biol. 2002 Dec.; 50(6):837-54).

Furthermore, according to the invention also anti-alpha-synuclein-antibody-inducing antigens based on nucleic acids ("aptamers") may be employed, and these, too, may be found with the most varying (oligonucleotide) libraries (e.g. with 2-180 nucleic acid residues) (e.g. Burgstaller et al., Curr. Opin. Drug Discov. Dev. 5(5) (2002), 690-700; Famulok et al., Acc. Chem. Res. 33 (2000), 591-599; Mayer et al., PNAS 98 (2001), 4961-4965, etc.). In anti-alpha-synuclein-antibody-inducing antigens based on nucleic acids, the nucleic acid backbone can be provided e.g. by the natural phosphor-diester compounds, or also by phosphorotioates or combinations or chemical variations (e.g. as PNA), wherein as bases, according to the invention primarily U, T, A, C, G, H and mC can be employed. The 2'-residues of the nucleotides which can be used according to the present invention preferably are H, OH, F, Cl, NH_2, O-methyl, O-ethyl, O-propyl or O-butyl, wherein the nucleic acids may also be differently modified, i.e. for instance with protective groups, as they are commonly employed in oligonucleotide synthesis. Thus, aptamer-based anti-alpha-synuclein-antibody-inducing antigens are also preferred anti-alpha-synuclein-antibody-inducing antigens within the scope of the present invention.

According to the present invention the term "synucleinopathy" includes all neurodegenerative disorders characterized by

pathological synuclein aggregations. Several neurodegenerative disorders including Parkinson's Disease (PD), Lewy Body Disease (LBD), Diffuse Lewy Body Disease (DLBD), Dementia with Lewy Bodies (DLB), Parkinsonism with Dementia (PDD), Multiple System Atrophy (MSA) and Neurodegeneration with Brain Iron Accumulation type I (NBIA Type I) are collectively grouped as synucleinopathies.

The peptides and polypeptides according to the present invention may be employed not only for treating synucleinopathies but also to prevent said diseases in individuals being at risk of developing a synucleinopathy (e.g. predisposed, for example genetically predisposed, to developing a synucleinopathy).

The abbreviations for the amino acid residues disclosed in the present invention follow the IUPAC recommendations:

Amino Acid	**3-Letter Code**	**1-Letter Code**
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic	Asp	D
Cysteine	Cys	C
Glutamic	Glu	E
Glutamine	Gln	Q
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W

Amino Acid	3-Letter Code	1-Letter Code
Tyrosine	Tyr	Y
Valine	Val	V

The peptides and polypeptides of the present invention may also be part of a polypeptide comprising 7 to 30 amino acid residues. Consequently n and m may independently be an integer selected from the group of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 20 and 25.

The at least one peptide or polypeptide according to the present invention may consist of the amino acid sequence $(X_1)_nX_2X_3X_4X_5GX_6P(X_7)_m$, wherein n and m are independently 0 or 1 or being part of a polypeptide which comprises at least 7 amino acid residues, preferably at least 10 amino acid residues, more preferably at least 15 amino acid residue, and/or a maximum of 50 amino acid residues, preferably a maximum of 30 amino acid residues, more preferably of 16 amino acid residues.

Surprisingly, it turned out that the compounds according to the present invention comprising or consisting of the amino acid sequences listed above are particularly suited to be used for the manufacture of a medicament to be used to treat or prevent synucleinopathies. These peptides (mimotopes) are able to induce the *in vivo* formation of antibodies directed to the original epitope of human alpha-synuclein comprising the amino acid sequence KNEEGAP and human alpha-synuclein protein itself. Said peptides (mimotopes) are, however, not able to induce immune reactivity against human beta-synuclein protein. The peptide induced antibodies are responsible for the removal of alpha-synuclein (which is involved in the formation of alpha-synuclein aggregates, Lewy bodies) and/or for the dissolution of alpha-synuclein aggregates (Lewy bodies) in an individual.

The peptides and polypeptides according to the present invention may be used for the preparation of a medicament, in particular a vaccine, which can be used to treat alpha-synucleinopathy, whereby the medicament is particularly suited to treat synucleinopathy selected from the group consisting of Parkinson's Disease (PD), Lewy Body Disease (LBD), Diffuse Lewy Body Disease (DLBD), Dementia with Lewy Bodies (DLB), Parkinson-

ism with Dementia (PDD), Multiple System Atrophy (MSA) and Neurodegeneration with Brain Iron Accumulation type I (NBIA Type I).

According to a preferred embodiment of the present invention the at least one peptide or polypeptide is coupled to a pharmaceutically acceptable carrier, preferably KLH (Keyhole Limpet Hemocyanin), tetanus toxoid, albumin-binding protein, bovine serum albumin, a dendrimer (MAP; Biol. Chem. 358: 581), peptide linkers (or flanking regions) as well as the adjuvant substances described in Singh et al., Nat. Biotech. 17 (1999), 1075-1081 (in particular those in Table 1 of that document), and O'Hagan et al., Nature Reviews, Drug Discovery 2 (9) (2003), 727-735 (in particular the endogenous immuno-potentiating compounds and delivery systems described therein), and others or mixtures thereof. The conjugation chemistry (e.g. via heterobifunctional compounds such as GMBS and of course also others as described in "Bioconjugate Techniques", Greg T. Hermanson) in this context can be selected from reactions known to the skilled man in the art. Moreover, the vaccine composition may be formulated with an adjuvant, preferably a low soluble aluminium composition, in particular aluminium hydroxide. Of course, also adjuvants like MF59 aluminium phosphate, calcium phosphate, cytokines (e.g., IL-2, IL-12, GM-CSF), saponins (e.g., QS21), MDP derivatives, CpG oligos, IC31, LPS, MPL, polyphosphazenes, emulsions (e.g., Freund's, SAF), liposomes, virosomes, iscoms, cochleates, PLG microparticles, poloxamer particles, virus-like particles, heat-labile enterotoxin (LT), cholera toxin (CT), mutant toxins (e.g., LTK63 and LTR72), microparticles and/or polymerized liposomes may be used.

The peptide or polypeptide of the present invention is preferably bound to the carrier or adjuvant via a linker, which is selected from the group consisting of NHS-poly (ethylene oxide) (PEO) (e.g. NHS-PEO_4-maleimide).

A vaccine which comprises the present compound (mimotope) and the pharmaceutically acceptable carrier may be administered by any suitable mode of application, e.g. i.d., i.p., i.m., intranasally, orally, subcutaneously, etc. and in any suitable delivery device (O'Hagan et al., Nature Reviews, Drug Discovery 2 (9), (2003), 727-735). The compound of the present invention is preferably formulated for subcutaneous, intradermal or intramus-

cular administration (see e.g. "Handbook of Pharmaceutical Manufacturing Formulations", Sarfaraz Niazi, CRC Press Inc, 2004).

Typically, the vaccine contains the compound according to the invention in an amount of from 0.1 ng to 10 mg, preferably 10 ng to 1 mg, in particular 100 ng to 100 µg, or, alternatively, e.g. 100 fmol to 10 µmol, preferably 10 pmol to 1 µmol, in particular 100 pmol to 100 nmol. Typically, the vaccine may also contain auxiliary substances, e.g. buffers, stabilizers etc.. Preferably, such auxiliary substances, e.g. a pharmaceutically acceptable excipient, such as water, buffer and/or stabilisers, are contained in an amount of 1 to 99 %(weight), more preferred 5 to 80%(weight), especially 10 to 70 %(weight). Possible administration regimes include a weekly, biweekly, fourweekly (monthly) or bimonthly treatment for about 1 to 12 months; however, also 2 to 5, especially 3 to 4, initial vaccine administrations (in one or two months), followed by boaster vaccinations 6 to 12 months thereafter or even years thereafter are preferred - besides other regimes already suggested for other vaccines. Another aspect of the present invention relates to a peptide having an amino acid sequence selected from the group consisting of $(X_1)_nKNDEGAP(X_7)_m$, $(X_1)_nANEEGAP(X_7)_m$, $(X_1)_nKAEEGAP(X_7)_m$, $(X_1)_nKNAEGAP(X_7)_m$, $(X_1)_nKNEAGAP(X_7)_m$, $(X_1)_nKNEEAAP(X_7)_m$, $(X_1)_nKNEEGAA(X_7)_m$, $(X_1)_nRNEEGAP(X_7)_m$, $(X_1)_nHNEEGAP(X_7)_m$, $(X_1)_nKNEDGAP(X_7)_m$, $(X_1)_nKQEEGAP(X_7)_m$, $(X_1)_nKSEEGAP(X_7)_m$, $(X_1)_nKNDDGAP(X_7)_m$, $(X_1)_nKPSFKNE(X_7)_m$, $(X_1)_nQPSFAME(X_7)_m$, $(X_1)_nSPSFKQE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, $(X_1)_nDPSFALE(X_7)_m$, $(X_1)_nLPSFRLE(X_7)_m$, $(X_1)_nEPNSRMD(X_7)_m$, $(X_1)_nQPSSKLD(X_7)_m$, $(X_1)_nHIHQSKFFDAPP(X_7)_m$, $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nQPAFAME(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nQPASKLD(X_7)_m$, $(X_1)_nQPSFAMA(X_7)_m$, $(X_1)_nTPSWKGA(X_7)_m$, $(X_1)_nQPSSKLA(X_7)_m$, $(X_1)_nAPSWKGE(X_7)_m$, $(X_1)_nTPSAKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKAE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, $(X_1)_nRNDEGAP(X_7)_m$, $(X_1)_nRNEDGAP(X_7)_m$, $(X_1)_nRQEEGAP(X_7)_m$, $(X_1)_nRSEEGAP(X_7)_m$, $(X_1)_nANDEGAP(X_7)_m$, $(X_1)_nANEDGAP(X_7)_m$, $(X_1)_nHSEEGAP(X_7)_m$, $(X_1)_nASEEGAP(X_7)_m$, $(X_1)_nHNEDGAP(X_7)_m$, $(X_1)_nHNDEGAP(X_7)_m$, $(X_1)_nRNAEGAP(X_7)_m$, $(X_1)_nHNAEGAP(X_7)_m$, $(X_1)_nKSAEGAP(X_7)_m$, $(X_1)_nKSDEGAP(X_7)_m$, $(X_1)_nKSEDGAP(X_7)_m$, $(X_1)_nRQDEGAP(X_7)_m$, $(X_1)_nRQEDGAP(X_7)_m$, $(X_1)_nHSAEGAP(X_7)_m$, $(X_1)_nRSAEGAP(X_7)_m$, $(X_1)_nRSDEGAP(X_7)_m$, $(X_1)_nRSEDGAP(X_7)_m$, $(X_1)_nHSDEGAP(X_7)_m$, $(X_1)_nHSEDGAP(X_7)_m$ and

$(X_1)_nRQDDGAP(X_7)_m$, especially an amino acid sequence selected from the group consisting of $(X_1)_nKNDEGAP(X_7)_m$, $(X_1)_nANEEGAP(X_7)_m$, $(X_1)_nKAEEGAP(X_7)_m$, $(X_1)_nKNAEGAP(X_7)_m$, $(X_1)_nRNEEGAP(X_7)_m$, $(X_1)_nHNEEGAP(X_7)_m$, $(X_1)_nKNEDGAP(X_7)_m$, $(X_1)_nKQEEGAP(X_7)_m$, $(X_1)_nKSEEGAP(X_7)_m$, $(X_1)_nKNDDGAP(X_7)_m$, $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, $(X_1)_nRNDEGAP(X_7)_m$, $(X_1)_nRNEDGAP(X_7)_m$, $(X_1)_nRQEEGAP(X_7)_m$, $(X_1)_nRSEEGAP(X_7)_m$, $(X_1)_nANDEGAP(X_7)_m$, $(X_1)_nANEDGAP(X_7)_m$, $(X_1)_nHSEEGAP(X_7)_m$, $(X_1)_nASEEGAP(X_7)_m$, $(X_1)_nHNEDGAP(X_7)_m$, $(X_1)_nHNDEGAP(X_7)_m$, $(X_1)_nRNAEGAP(X_7)_m$, $(X_1)_nHNAEGAP(X_7)_m$, $(X_1)_nKSAEGAP(X_7)_m$, $(X_1)_nKSDEGAP(X_7)_m$, $(X_1)_nKSEDGAP(X_7)_m$, $(X_1)_nRQDEGAP(X_7)_m$, $(X_1)_nRQEDGAP(X_7)_m$, $(X_1)_nHSAEGAP(X_7)_m$, $(X_1)_nRSAEGAP(X_7)_m$, $(X_1)_nRSDEGAP(X_7)_m$, $(X_1)_nRSEDGAP(X_7)_m$, $(X_1)_nHSDEGAP(X_7)_m$, $(X_1)_nHSEDGAP(X_7)_m$ and $(X_1)_nRQDDGAP(X_7)_m$, wherein X_1 and X_7 is cysteine and n and m, independently, are 0 or 1.

According to a preferred embodiment of the present invention the peptide is coupled to a pharmaceutically acceptable carrier, preferably KLH (Keyhole Limpet Hemocyanin).

The pharmaceutical formulation according to the present invention, which can be formulated as a vaccine for, e.g., subcutaneous, intradermal and/or intramuscular administration, may be used in the treatment of any kind of synucleinopathy.

The present invention is further illustrated in the following figures and examples, however, without being restricted thereto.

Fig.1 shows the sequence of full length alpha-synuclein (140aa; swiss prot entry: P37840) and the sequence used to create a monoclonal antibody for detection of full length alpha-synuclein as well as C-terminally truncated and modified versions thereof. The peptide at position 100-109 used for generation of the monoclonal antibody is underlined. The peptide (p4453) was coupled to a C at C-terminal position.

Fig.2 shows detection of alpha-synuclein by ELISA using the generated monoclonal antibody specific for human alpha-synuclein at position 100-109. The monoclonal antibody 12-9-9 was generated and tested for its specificity to synucleins in ELISA. Alpha-synuclein (p4446) and p4453 the human epitope are detected. The negative control protein p4447 (beta-synuclein) is not detected.

Fig. 3 shows the definition of the minimal epitope of the monoclonal antibody 12-9-9 by ELISA.

The peptides p4446 (alpha-synuclein), p4453(human epitope used to create the antibody tested) are detected by the antibody. The original epitope p4453 was N- or C-terminally truncated and used for ELISA to define the minimal epitope required for specific binding. The peptides p5399 and p5403 lost binding to the monoclonal antibody 12-9-9. Thus the minimal sequence needed for binding of 12-9-9 is predicted as KNEEGAP located at position 102-108 of alpha-synuclein, while truncation of one of the flanking amino acids abolished the binding. Data are presented in a linear scale.

Fig. 4 shows detection of epitopes and mimotopes by ELISA using a monoclonal specific for human alpha-synuclein at position 100-109. Alpha-Synuclein as well as the peptides p5436 (human minimal epitope) and the mimotopes p5439 are detected similarly by the monoclonal antibody 12-9-9. The mimotope p5440 is not detected, while mimotope p5444 is detected much weaker than the human epitope by the monoclonal antibody 12-9-9.

Fig. 5 shows the induction of immune response against alpha-synuclein after peptide immunization. Sera of immunized mice (p5436 to p5590) show titers against alpha-synuclein after 3 vaccinations. Sera of immunized mice (p5463 to p5466) do not detect alpha synuclein (Titers measured in ELISA are around or below 1:50 half-max). Class of immunogenicity was defined as follows: Class 2: peptides inducing an immune response with OD halfmax higher than 1:1000. Class 1: peptides inducing an immune response with OD halfmax between 1000 and 51. Class 0: peptides inducing no or an very low immune resonse with OD halfmax around 50 or lower.

EXAMPLES:

To identify peptides and polypeptides which can be used to treat and/or prevent synucleopathies an antibody was used, which is able to detect the human alpha-synuclein-derived amino acid sequence LGKNEEGAPQ (= original epitope, SEQ ID No. 3, p4453) and full length human alpha-synuclein (SEQ ID No. 1, p4446). It does not recognize human beta-synuclein (SEQ ID No. 2, p4447;accession number Q16143: mdvfmkglsm akegvvaaae ktkqgvteaa ektkegvlyv gsktregvvq gvasvaektk eqashlggav fsgagniaaa

tglvkreefp tdlkpeevaq eaaeepliep lmepegesye dppqeeyqey epea). The antibody may be a monoclonal or polyclonal antibody preparation or any antibody part or derivative thereof and binds specifically to the LGKNEEGAPQ epitope of human alpha-synuclein, i. e. it does bind to peptide and full length protein but does not bind to human beta-synuclein. The mimotopes are identified and further characterised with such monoclonal antibodies (detecting a sequence within amino acids 100-109 of the human alpha-synuclein protein) and peptide libraries.

Example 1: Generation of monoclonal antibodies to specifically detect original human alpha-synuclein epitope LGKNEEGAPQC SEQ ID No. 3, p4453 *and human alpha-synuclein but not human beta-synuclein.*

A monoclonal antibody derived from the fusion "AFFiRiS 6": Balb/c mice (Charles River) were immunized with original alpha-synuclein epitope LGKNEEGAPQ-C coupled to BTG (bovine thyroglobuline) and CFA (complete Freund´s adjuvant; first injection) as well as IFA (incomplete Freund´s adjuvant; 3 booster injections) as adjuvant. LGKNEEGAPQ- peptide-specific, antibody-producing hybridomas are detected by ELISA (LGKNEEGAPQC-peptide-coated ELISA plates). Human alpha-synuclein (recombinant protein, p4446) is used as positive control peptide: hybridomas recognizing the recombinant protein immobilized on ELISA plates are included because they are binding both peptide and full length alpha-synuclein specifically. Human beta-synuclein (recombinant protein, p4447) is used as negative control peptide: hybridomas recognizing both recombinant proteins immobilized on ELISA plates are excluded because they do not distinguish between the two different synuclein proteins. The Hybridoma clone (12-9-9; IgG1, kappa) was analysed for specific detection of the natural human alpha-synuclein epitope LGKNEEGAPQ. 12-9-9 recognizes the injected epitope as well as full length alpha-synuclein protein (recombinant protein; obtained from rPeptide, Bogart, GA, USA) in ELISA (see Fig. 2). It however does not detect beta-synuclein protein (recombinant protein, obtained from rPeptide, Bogart, GA, USA) in ELISA (see Fig. 2). Subsequently, the minimal epitope required for binding of the antibody has been defined by ELISA using the peptides *p4446, p4453, p5397, p5398, p5399, p5400, p5401, p5402, p5403, p5404, p5405, p5406*

(see Fig. 3) and p5436 (see Fig.4). p4446, p4453, p5397, p5398 and p5402 *as well as p5436* retained full binding capacities whereas *p5399, p5400, p5401, p5403, p5404, p5405 and p5406 lost binding to 12-9-9. Thus the minimal required epitope for binding has been defined as KNEEGAP.*

Example 2: Phage Display, in vitro binding and inhibition ELISA

Phage Display libraries used in this example were: Ph.D. 7: New England BioLabs E8102L (linear 7mer library), Ph.D. 12: New England BioLabs E8111L (linear 12mer library) and Ph.D. C7C: New England BioLabs E8120L (a disulfide-constrained heptapeptide library) Phage Display was done according to manufacturer's protocol (www.neb.com). After 2 or 3 subsequent rounds of panning, single phage clones were picked and phage supernatants were subjected to ELISA on plates coated with the antibody that was used for the panning procedure. Phage clones that were positive in this ELISA (strong signal for the target, but no signal for unspecific control) were sequenced. From DNA sequences, peptide sequences were deduced. These peptides were synthesized and characterised in binding and inhibition ELISA. To some peptides additional AA were attached to the C-terminus. Additionally, some novel mimotopes were created by combining sequence information from mimotopes identified in the screen. Both groups containing newly designed mimotopes were used to support the identification of a consensus sequence for a mimotope vaccination.

1. In vitro binding assay (ELISA)

Peptides derived from Phage Display as well as N-terminally truncated variants thereof were coupled to BSA and bound to ELISA plates (1µM) and subsequently incubated with the monoclonal antibody that was used for the screening procedure to analyse binding capacity of identified peptides (see Fig. 4).

2. In vitro inhibition assay (ELISA)

Different amounts of peptides (concentrations ranging from 400µg/ml to 3µg/ml (serial dilutions), derived from Phage Display were incubated with the monoclonal antibody that was used for the screening procedure. Peptides diminishing subsequent binding of the antibody to the original human alpha-synuclein epitope

(p5436) and the human alpha-synuclein protein (p4446) coated on ELISA plates were considered as inhibiting in this assay.

Example 3: in vivo testing of mimotopes: analysis of immunogenicity

1.In vivo testing of mimotopes

Inhibiting as well as non-inhibiting peptides were coupled to KLH and injected into mice (wildtype C57/Bl6 or BalbC mice; subcutaneous injection into the flank) together with an appropriate adjuvant (aluminium hydroxide). Animals were vaccinated 3 times in biweekly intervals and sera were taken biweekly as well. Titers to injected peptides as well as to an irrelevant peptide were determined with every serum. Titers against the recombinant human alpha-synuclein protein and recombinant human beta-synuclein were determined starting with Serum 2 respectively. In general sera were analysed by reaction against peptides coupled to Bovine Serum Albumin (BSA) and recombinant full length proteins which were immobilised on ELISA plates. Titers were determined using anti mouse IgG specific antibodies. For examples of immunogenicity against injected peptide and alpha-synuclein see Table 5 and Table 6.

2. Results

2.1. Identification of an alpha-synuclein specific mAB: Figure 2 depicts the characterisation of the alpha-synuclein specific monoclonal antibody 12-9-9 (IgG1, kappa) derived from fusion Affiris 6.

2.2. Screening for alpha-synuclein specific Mimotopes:

2.2.1. Phage Display PhD 7, PhD12 and PhD C7C and mutational Screen

2.2.1.1. Screening with monoclonal antibody directed against LGKNEEGAPQ

By screening of PhD 7, PhD12 and PhD C7C phage display libraries and selective displacement of single amino acids a total of 60 sequences was identified (see Table 1; ID18-77).

Table 1 shows examples all peptides used.

SEQ ID	peptide No	sequence
1	p4446	Alpha-synuclein (Fig. 1; P37840)
2	p4447	Beta-Synuclein;Q16143
3	p4453	LGKNEEGAPQC
4	p4454	MGKGEEGYPQC
5	p5397	GKNEEGAPQC
6	p5398	KNEEGAPQC
7	p5399	NEEGAPQC
8	p5400	EEGAPQC
9	p5401	EGAPQC
10	p5402	LGKNEEGAPC
11	p5403	LGKNEEGAC
12	p5404	LGKNEEGC
13	p5405	LGKNEEC
14	p5406	LGKNEC
15	p5435	CKNEEGAP
16	p5436	KNEEGAPC
17	p1253	DAEFRHDSGY-C
18	p5437	ANEEGAPC
19	p5438	KAEEGAPC
20	p5439	KNAEGAPC
21	p5440	KNEAGAPC
22	p5441	KNEEAAPC
23	p5442	KNEEGAAC
24	p5443	RNEEGAPC
25	p5444	HNEEGAPC
26	p5445	KNDEGAPC
27	p5446	KNEDGAPC
28	p5447	KQEEGAPC
29	p5448	KSEEGAPC
30	p5449	KNDDGAPC
31	p5461	KPSFKNEC
32	p5462	QPSFAMEC

33	p5463	SPSFKQEC
34	p5464	TPSWKGEC
35	p5465	DPSFALEC
36	p5466	LPSFRLEC
37	p5467	EPNSRMDC
38	p5468	QPSSKLDC
39	p5469	HIHQSKFFDAPPC
40	p5547	QASFAMEC
41	p5548	TASWKGEC
42	p5549	QASSKLDC
43	p5550	QPAFAMEC
44	p5551	TPAWKGEC
45	p5552	QPASKLDC
46	p5553	QPSFAMAC
47	p5554	TPSWKGAC
48	p5555	QPSSKLAC
49	p5556	APSWKGEC
50	p5557	TPSAKGEC
51	p5558	TPSWAGEC
52	p5559	TPSWKAEC
53	p5560	CTPSWKGE
54	p5587	RNDEGAPC
55	p5588	RNEDGAPC
56	p5589	RQEEGAPC
57	p5590	RSEEGAPC
58	p5591	ANDEGAPC
59	p5592	ANEDGAPC
60	p5593	HSEEGAPC
61	p5594	ASEEGAPC
62	p5595	HNEDGAPC
63	p5596	HNDEGAPC
64	p5597	RNAEGAPC
65	p5598	HNAEGAPC
66	p5599	KSAEGAPC
67	p5600	KSDEGAPC

68	p5601	KSEDGAPC
69	p5602	RQDEGAPC
70	p5603	RQEDGAPC
71	p5604	HSAEGAPC
72	p5605	RSAEGAPC
73	p5606	RSDEGAPC
74	p5607	RSEDGAPC
75	p5608	HSDEGAPC
76	p5609	HSEDGAPC
77	p5610	RQDDGAPC

Table 2 shows examples of peptides and their binding capacity as compared to the original epitope.

internal number	**Sequence**	**Binding**	**remark**
p5435	CKNEEGAP	2	original
p5436	KNEEGAPC	2	original
p5437	ANEEGAPC	2	mimotope
p5438	KAEEGAPC	1	mimotope
p5439	KNAEGAPC	2	mimotope
p5443	RNEEGAPC	2	mimotope
p5444	HNEEGAPC	2	mimotope
p5445	KNDEGAPC	2	mimotope
p5446	KNEDGAPC	2	mimotope
p5447	KQEEGAPC	2	mimotope
p5448	KSEEGAPC	2	mimotope
p5449	KNDDGAPC	2	mimotope
p5398	KNEEGAPQC	2	original
p5402	LGKNEEGAPC	2	original
p5397	GKNEEGAPQC	2	original

p4454	MGKGEEGYPQC	2	original mouse
p4453	LGKNEEGAPQC	2	original
p5461	KPSFKNEC	2	mimotope
p5462	QPSFAMEC	2	mimotope
p5463	SPSFKQEC	2	mimotope
p5464	TPSWKGEC	2	mimotope
p5465	DPSFALEC	2	mimotope
p5466	LPSFRLEC	1	mimotope
p5468	QPSSKLDC	2	mimotope
p5547	QASFAMEC	1	mimotope
p5548	TASWKGEC	2	mimotope
p5549	QASSKLDC	1	mimotope
p5551	TPAWKGEC	1	mimotope
p5556	APSWKGEC	2	mimotope
p5558	TPSWAGEC	2	mimotope
p5560	CTPSWKGE	2	mimotope
p5587	--RNDEGAPC	2	mimotope
p5588	--RNEDGAPC	2	mimotope
p5589	--RQEEGAPC	2	mimotope
p5590	--RSEEGAPC	2	mimotope
p5591	--ANDEGAPC	2	mimotope
p5593	--HSEEGAPC	1	mimotope
p5594	--ASEEGAPC	1	mimotope
p5596	--HNDEGAPC	2	mimotope
p5597	--RNAEGAPC	2	mimotope
p5598	--HNAEGAPC	1	mimotope
p5599	--KSAEGAPC	2	mimotope
p5600	--KSDEGAPC	2	mimotope
p5601	--KSEDGAPC	2	mimotope

p5602	--RQDEGAPC	2	mimotope
p5603	--RQEDGAPC	2	mimotope
p5604	--HSAEGAPC	1	mimotope
p5605	--RSAEGAPC	2	mimotope
p5606	--RSDEGAPC	2	mimotope
p5607	--RSEDGAPC	2	mimotope
p5608	--HSDEGAPC	1	mimotope
p5610	--RQDDGAPC	2	mimotope
p4446		2	α-Syn

Table 2: Examples of alpha-synuclein epi- and mimotopes binding to the monoclonal Antibody 12-9-9

the binding capacity is coded by the following code:

0: no binding to 12-9-9 detectable in ELISA

1: weak binding: binding of mimotope weaker compared to the minimal original sequence p5436

2: strong binding: binding of mimotope similar to the minimal original sequence p5436

2.3. In vitro characterisation of mimotopes identified in a screen (Phage Display and peptide screening) with a monoclonal antibody directed against alpha-synuclein:

Fig. 2 and 3 show representative examples for binding and inhibition assays used to characterise mimotopes in vitro. Data obtained are summarised in Tables 2 and 3 respectively.

Table 3: Inhibition assay

designation	**Sequence**	**Competition**	**remark**
p5435	CKNEEGAP	1	original
p5436	KNEEGAPC	2	original
p5439	KNAEGAPC	1	mimotope

p5443	RNEEGAPC	2	mimotope
p5445	KNDEGAPC	2	mimotope
p5446	KNEDGAPC	1	mimotope
p5448	KSEEGAPC	1	mimotope
p5449	KNDDGAPC	1	mimotope
p5398	KNEEGAPQC	2	original
p5402	LGKNEEGAPC	2	original
p5397	GKNEEGAPQC	2	original mouse
p5464	TPSWKGEC	1	mimotope
p5548	TASWKGEC	1	mimotope
p5556	APSWKGEC	1	mimotope
p5557	TPSAKGEC	1	mimotope
p5587	RNDEGAPC	2	mimotope
p5588	RNEDGAPC	1	mimotope
p5590	RSEEGAPC	1	mimotope
p5597	RNAEGAPC	1	mimotope
p5600	KSDEGAPC	1	mimotope
p5602	RQDEGAPC	1	mimotope
p5603	RQEDGAPC	1	mimotope

Table 3: Alpha-synuclein mimotopes identified in this invention giving positive results in inhibition assays

Legend to Table 3: the competition capacity is coded by the following code:

0: no competition of 12-9-9 detectable in ELISA

1: weak competition: competition of mimotope weaker compared to the minimal original sequence p5436

2: strong competition: competition of mimotope similar to the minimal original sequence p5436

Table 4: Non-Mimotope peptides and proteins:

SEQ ID No.	designation	sequence
1	p4446	Alpha-synuclein
2	p4447	Beta-Synuclein
3	p4453	LGKNEEGAPQC
4	p4454	MGKGEEGYPQC
5	p5397	GKNEEGAPQC
6	p5398	KNEEGAPQC
7	p5399	NEEGAPQC
8	p5400	EEGAPQC
9	p5401	EGAPQC
10	p5402	LGKNEEGAPC
11	p5403	LGKNEEGAC
12	p5404	LGKNEEGC
13	p5405	LGKNEEC
14	p5406	LGKNEC
15	p5435	CKNEEGAP
16	p5436	KNEEGAPC
17	p1253	DAEFRHDSGY-C

2.4. *In vivo* characterisation of mimotopes identified in screening Phage Display Libraries with a monoclonal antibody directed against alpha-synuclein:

Female C57/Bl6 mice or BalbC, 5-6 mice per group, were subcutaneously immunized with 30 µg peptide coupled to KLH. Control groups were injected with PBS or the original epitope. As adjuvant alum was used. The peptides administered were all able to bind to monoclonal antibodies specifically binding aa100-109 of human alpha-synuclein although some of the peptides did inhibit the binding of the original epitope to its parental antibody in vitro only weakly (in an in vitro inhibition assay). The in vi-

tro ELISA assay to determine the antibody titer was performed with sera of single mice (see Tab. 5) after each vaccination in a two week interval. The wells of the ELISA plate were coated with mimotope-BSA conjugates. The positive control was performed by reaction of the parental antibody with the respective mimotope-BSA conjugate. The detection was performed with anti-mouse IgG. Additionally, recombinant proteins were immobilised on ELISA plates and sera reacted accordingly. For all mimotopes tested in C57/Bl6 mice or BalbC, antibodies reacting to the individual injected peptide could be detected after repeated vaccination. Although not all mice induced higher titer against alpha-synuclein (see tab. 5 for examples).

Table 5: Induction of immune response is indicated by the titer against injected peptide (p4446). Titer was measured by ELISA and indicated as OD halfmax.

designation	**Sequence**	**Titer in animals OD halfmax**	**remark**
		injected pep.	
p5436	KNEEGAPC	10000	original
p5439	KNAEGAPC	8000	mimotope
p5443	RNEEGAPC	9000	mimotope
p5445	KNDEGAPC	26000	mimotope
p5402	LGKNEEGAPC	20000	original
p4454	MGKGEEGYPQC	17000	original mouse

Table 6: class of immunogenicity of mimotopes against a Syn

designation	sequence	class of immunogenicity: a Syn	remark
p5402	LGKNEEGAP-C	2	original
p5436	KNEEGAPC	2	original
p5445	KNDEGAPC	2	mimotope
p5443	RNEEGAPC	1	mimotope
p5587	RNDEGAPC	1	mimotope
p5439	KNAEGAPC	1	mimotope

p5600	KSDEGAPC	1	mimotope
p4454	MGKGEEGYPQC	1	original mouse
p5597	RNAEGAPC	1	mimotope
p5590	RSEEGAPC	1	mimotope
p5463	SPSFKQEC	0	mimotope
p5556	APSWKGEC	0	mimotope
p5462	QPSFAMEC	0	mimotope
p5464	TPSWKGEC	0	mimotope
p5468	QPSSKLDC	0	mimotope
p5461	KPSFKNEC	0	mimotope
p5465	DPSFALEC	0	mimotope
p5466	LPSFRLEC	0	mimotope

Class of immunogenicity:
peptides were ranked according to their capacity to induce an immune response
2: peptides inducing an immune response with OD halfmax higher than 1000.
1: peptides inducing an immune response with OD halfmax between 1000 and 51.
0: peptides inducing no immune response or a very low immune response with OD halfmax around 50 or lower.

Claims:

1. Composition comprising at least one peptide or polypeptide comprising the amino acid sequence

$(X_1)_nX_2X_3X_4X_5GX_6P(X_7)_m$ (Formula I),

wherein

X_1 is any amino acid residue,
X_2 is an amino acid residue selected from the group consisting of lysine (K), arginine (R), alanine (A) and histidine (H),
X_3 is an amino acid residue selected from the group consisting of asparagine (N), glutamine (Q), serine (S), glycine (G) and alanine (A), preferably asparagine (N), serine (S), glycine (G) and alanine (A),
X_4 is an amino acid residue selected from the group consisting of glutamic acid (E), aspartic acid (D) and alanine (A),
X_5 is an amino acid residue selected from the group consisting of glutamic acid (E) and aspartic acid (D),
X_6 is an amino acid residue selected from the group consisting of alanine (A) and tyrosine (Y),
X_7 is any amino acid residue,
n and m, independently, are 0 or an integer of more than 0,

and wherein the amino acid sequence according to Formula I is not identical with, or does not comprise the 7-mer polypeptide fragment of alpha-synuclein having the amino acid sequence KNEEGAP,

said at least one peptide or polypeptide having a binding capacity to an antibody which is specific for an epitope of alpha-synuclein comprising the amino acid sequence KNEEGAP

for use in preventing and/or treating synucleinopathies.

2. Composition according to claim 1, wherein X_2 is an amino acid residue selected from the group consisting of lysine (K) and arginine (R) and/or X_6 is alanine (A).

3. Composition according to claim 1 or 2 characterised in that the peptide or polypeptide comprises an amino acid sequence selected from the group consisting of $(X_1)_nKNDEGAP(X_7)_m$, $(X_1)_nANEEGAP(X_7)_m$, $(X_1)_nKAEEGAP(X_7)_m$, $(X_1)_nKNAEGAP(X_7)_m$, $(X_1)_nRNEEGAP(X_7)_m$, $(X_1)_nHNEEGAP(X_7)_m$, $(X_1)_nKNEDGAP(X_7)_m$, $(X_1)_nKQEEGAP(X_7)_m$, $(X_1)_nKSEEGAP(X_7)_m$, $(X_1)_nKNDDGAP(X_7)_m$, $(X_1)_nRNDEGAP(X_7)_m$, $(X_1)_nRNEDGAP(X_7)_m$, $(X_1)_nRQEEGAP(X_7)_m$, $(X_1)_nRSEEGAP(X_7)_m$, $(X_1)_nANDEGAP(X_7)_m$, $(X_1)_nANEDGAP(X_7)_m$, $(X_1)_nHSEEGAP(X_7)_m$, $(X_1)_nASEEGAP(X_7)_m$, $(X_1)_nHNEDGAP(X_7)_m$, $(X_1)_nHNDEGAP(X_7)_m$, $(X_1)_nRNAEGAP(X_7)_m$, $(X_1)_nHNAEGAP(X_7)_m$, $(X_1)_nKSAEGAP(X_7)_m$, $(X_1)_nKSDEGAP(X_7)_m$, $(X_1)_nKSEDGAP(X_7)_m$, $(X_1)_nRQDEGAP(X_7)_m$, $(X_1)_nRQEDGAP(X_7)_m$, $(X_1)_nHSAEGAP(X_7)_m$, $(X_1)_nRSAEGAP(X_7)_m$, $(X_1)_nRSDEGAP(X_7)_m$, $(X_1)_nRSEDGAP(X_7)_m$, $(X_1)_nHSDEGAP(X_7)_m$, $(X_1)_nHSEDGAP(X_7)_m$, $(X_1)_nRQDDGAP(X_7)_m$, preferably $(X_1)_nKNDEGAP(X_2)_m$, $(X_1)_nRNEEGAP(X_2)_m$, $(X_1)_nRNDEGAP(X_2)_m$, $(X_1)_nKNAEGAP(X_2)_m$, $(X_1)_nKSDEGAP(X_2)_m$, $(X_1)_nRNAEGAP(X_2)_m$ or $(X_1)_nRSEEGAP(X_2)_m$.

4. Composition comprising at least one peptide or polypeptide comprising an amino acid sequence selected from the group consisting of $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, wherein

X_1 is any amino acid residue,

X_7 is any amino acid residue,

n and m, independently, are 0 or an integer of more than 0,

said at least one peptide or polypeptide having a binding capacity to an antibody which is specific for an epitope of alpha-synuclein comprising the amino acid sequence KNEEGAP

for use in preventing and/or treating synucleinopathies.

5. Composition according to any one of claims 1 to 4, characterised in that n and/or m are 1 and X_1 and/or X_7 are cysteine (C).

6. Composition according to any one of claims 1 to 5, characterised in that the peptide or polypeptide comprises 7 to 30, preferably 7 to 20, more preferably 7 to 16, most preferably 8, amino acid residues.

7. Composition according to any one of claims 1 to 6, character-

ised in that the synucleinopathy is selected from the group consisting of Lewy Body Disorders (LBDs), preferably Parkinson's Disease (PD), Parkison's Disease with Dementia (PDD) and Dementia with Lewy Bodies (DLB), as well as Multiple System Atrophy (MSA) or Neurodegeneration with Brain Iron Accumulation type I (NBIA Type I).

8. Composition according to any one of claims 1 to 7, characterised in that the at least one peptide or polypeptide is coupled to a pharmaceutically acceptable carrier, preferably KLH (Keyhole Limpet Hemocyanin).

9. Composition according to any one of claims 1 to 8, characterised in that the at least one peptide or polypeptide is formulated for intravenous, subcutaneous, intradermal or intramuscular administration.

10. Composition according to any one of claims 1 to 9, characterised in that the at least one peptide or polypeptide is formulated with an adjuvant, preferably aluminium hydroxide.

11. Composition according to any one of claims 1 to 10, characterised in that the at least one peptide or polypeptide is contained in the medicament in an amount of from 0.1 ng to 10 mg, preferably 10 ng to 1 mg, in particular 100 ng to 100 µg.

12. Peptide having an amino acid sequence selected from the group consisting of $(X_1)_nKNDEGAP(X_7)_m$, $(X_1)_nANEEGAP(X_7)_m$, $(X_1)_nKAEEGAP(X_7)_m$, $(X_1)_nKNAEGAP(X_7)_m$, $(X_1)_nRNEEGAP(X_7)_m$, $(X_1)_nHNEEGAP(X_7)_m$, $(X_1)_nKNEDGAP(X_7)_m$, $(X_1)_nKQEEGAP(X_7)_m$, $(X_1)_nKSEEGAP(X_7)_m$, $(X_1)_nKNDDGAP(X_7)_m$, $(X_1)_nQASFAME(X_7)_m$, $(X_1)_nTASWKGE(X_7)_m$, $(X_1)_nQASSKLD(X_7)_m$, $(X_1)_nTPAWKGE(X_7)_m$, $(X_1)_nTPSWAGE(X_7)_m$, $(X_1)_nTPSWKGE(X_7)_m$, $(X_1)_nRNDEGAP(X_7)_m$, $(X_1)_nRNEDGAP(X_7)_m$, $(X_1)_nRQEEGAP(X_7)_m$, $(X_1)_nRSEEGAP(X_7)_m$, $(X_1)_nANDEGAP(X_7)_m$, $(X_1)_nANEDGAP(X_7)_m$, $(X_1)_nHSEEGAP(X_7)_m$, $(X_1)_nASEEGAP(X_7)_m$, $(X_1)_nHNEDGAP(X_7)_m$, $(X_1)_nHNDEGAP(X_7)_m$, $(X_1)_nRNAEGAP(X_7)_m$, $(X_1)_nHNAEGAP(X_7)_m$, $(X_1)_nKSAEGAP(X_7)_m$, $(X_1)_nKSDEGAP(X_7)_m$, $(X_1)_nKSEDGAP(X_7)_m$, $(X_1)_nRQDEGAP(X_7)_m$, $(X_1)_nRQEDGAP(X_7)_m$, $(X_1)_nHSAEGAP(X_7)_m$, $(X_1)_nRSAEGAP(X_7)_m$, $(X_1)_nRSDEGAP(X_7)_m$, $(X_1)_nRSEDGAP(X_7)_m$, $(X_1)_nHSDEGAP(X_7)_m$,

$(X_1)_nHSEDGAP(X_7)_m$ and $(X_1)_nRQDDGAP(X_7)_m$, wherein X_1 and X_7 is cysteine and n and m, independently, are 0 or 1.

13. Peptide according to claim 12, characterised in that the peptide is coupled to a pharmaceutically acceptable carrier, preferably KLH (Keyhole Limpet Hemocyanin).

14. Peptide according to claim 12 or 13 for preventing and/or treating synucleinopathies.

15. Pharmaceutical formulation, preferably a vaccine, comprising at least one peptide according to any one of claims 12 to 14.

Alpha-synuclein protein sequence:

```
        10         20         30         40         50         60
MDVFMKGLSK AKEGVVAAAE KTKQGVAEAA GKTKEGVLYV GSKTKEGVVH GVATVAEKTK
        70         80         90        100        110        120
EQVTNVGGAV VTGVTAVAQK TVEGAGSIAA ATGFVKKDQL GKNEEGAPQE GILEDMPVDP
       130        140
DNEAYEMPSE EGYQDYEPEA
```

Fig. 1

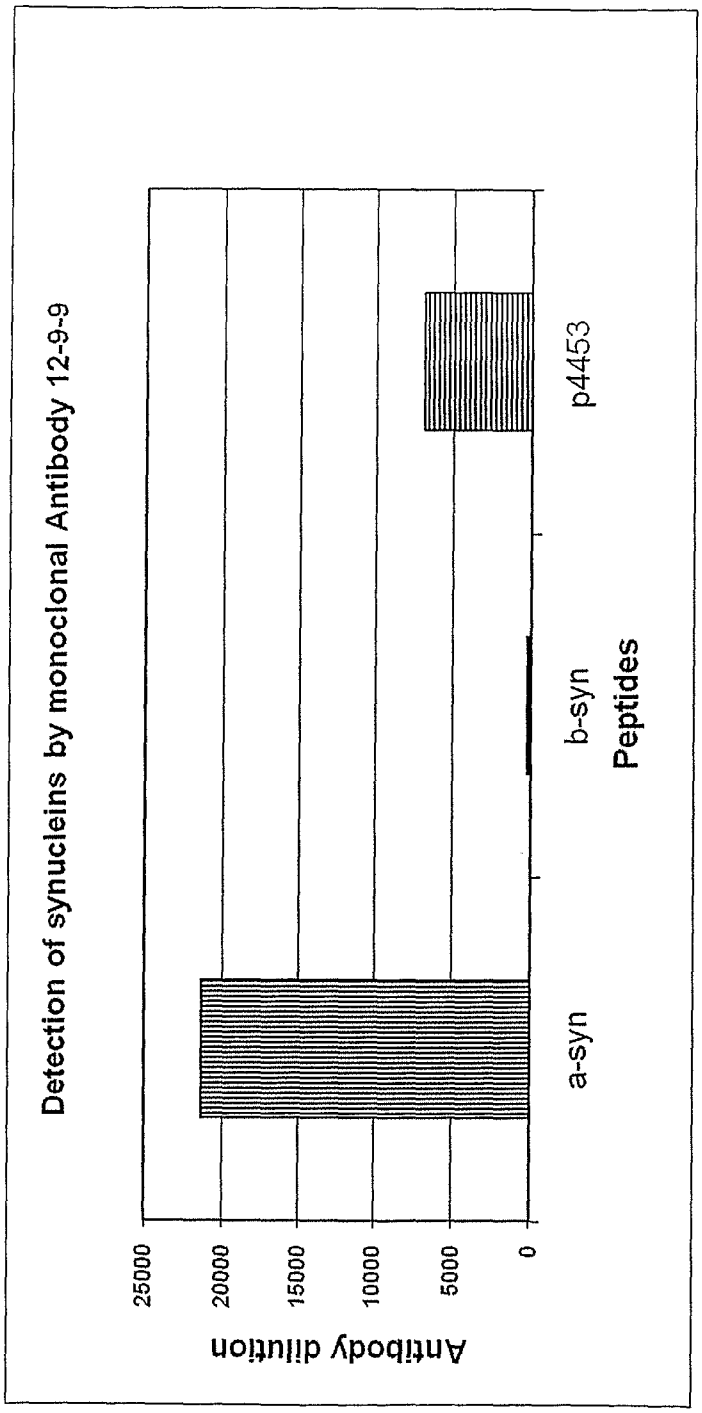
Detection of synucleins by monoclonal Antibody 12-9-9
Antibody dilution
25000
20000
15000
10000
5000
0
a-syn
b-syn
p4453
Peptides

Fig. 2

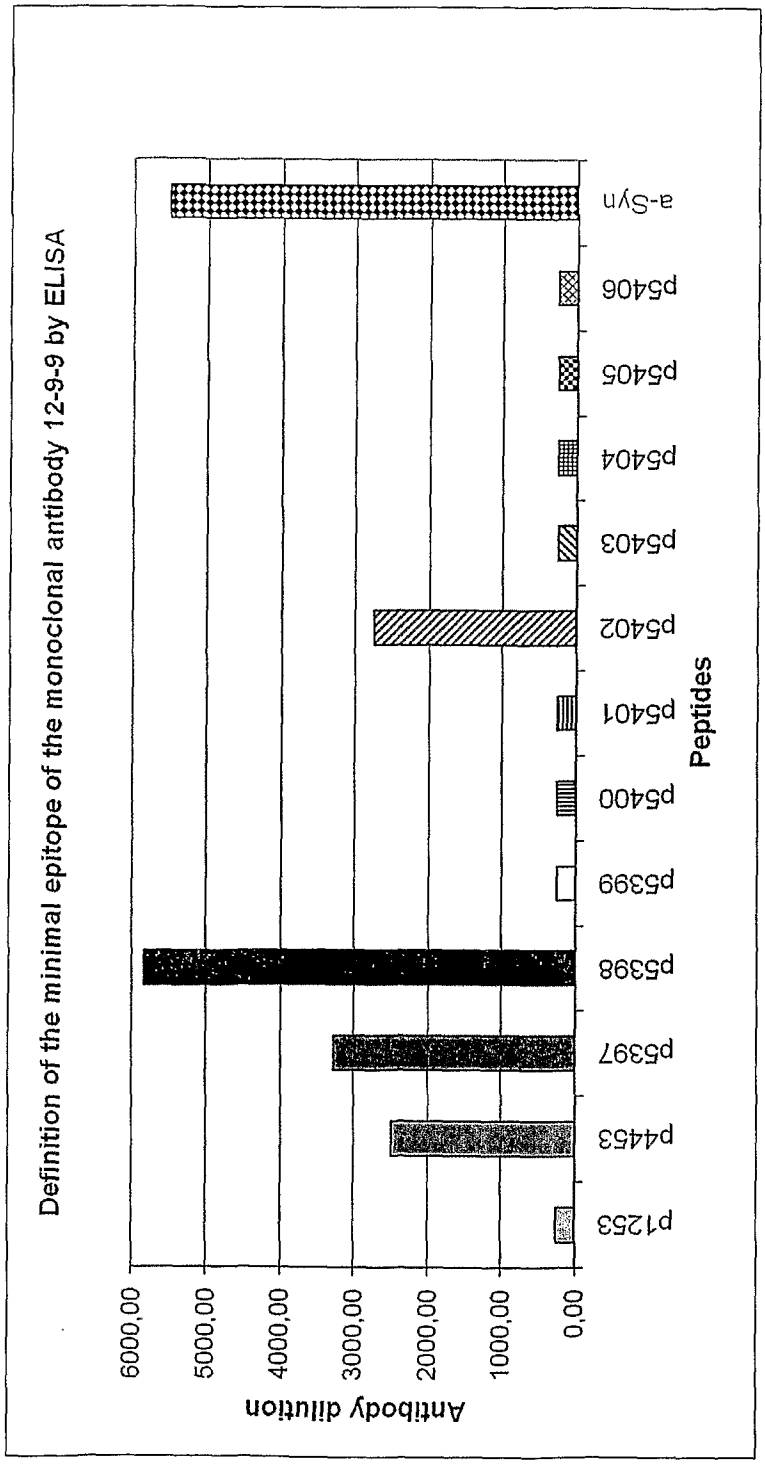
Definition of the minimal epitope of the monoclonal antibody 12-9-9 by ELISA
Antibody dilution
6000,00
5000,00
4000,00
3000,00
2000,00
1000,00
0,00
p1253
p4453
p5397
p5398
p5399
p5400
p5401
p5402
p5403
p5404
p5405
p5406
a-Syn
Peptides

Fig. 3

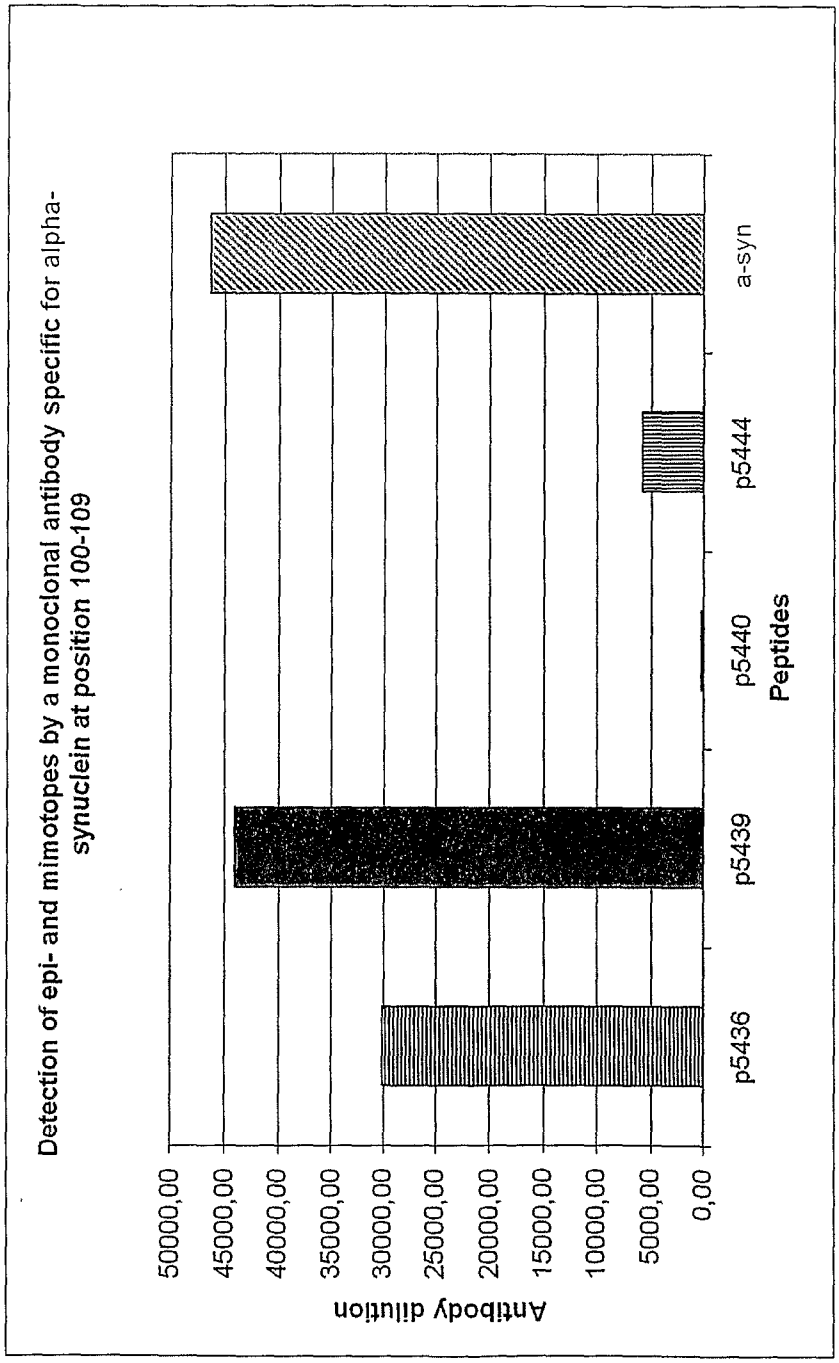
Detection of epi- and mimotopes by a monoclonal antibody specific for alpha-synuclein at position 100-109
Antibody dilution
50000,00
45000,00
40000,00
35000,00
30000,00
25000,00
20000,00
15000,00
10000,00
5000,00
0,00
p5436
p5439
p5440
p5444
a-syn
Peptides

Fig. 4

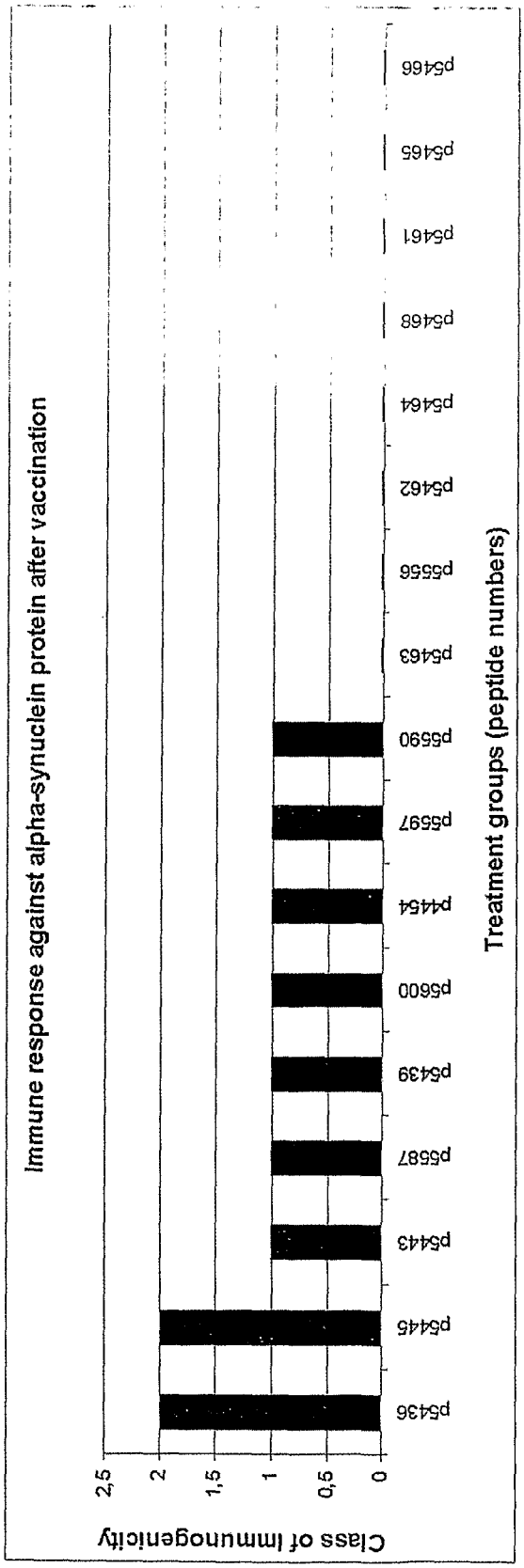
Immune response against alpha-synuclein protein after vaccination
Class of immunogenicity
2,5
2
1,5
1
0,5
0
Treatment groups (peptide numbers)
p5436
p5445
p5443
p5587
p5439
p5600
p4454
p5597
p5590
p5463
p5556
p5462
p5464
p5468
p5461
p5465
p5466

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/AT2010/000303

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K38/17 A61K39/00 C07K7/06 A61P25/16 A61P25/28

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61K C07K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, WPI Data, BIOSIS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2004/041067 A2 (ELAN PHARM INC [US]; UNIV CALIFORNIA [US]) 21 May 2004 (2004-05-21) see claims 1-16 and pages 59-61	4-15
Y	US 2008/014194 A1 (SCHENK DALE B [US] ET AL) 17 January 2008 (2008-01-17) see claim 1, Seq.15 and [0277]	4-15
A	WO 2006/045037 A2 (ELAN PHARM INC [US]; CHILCOTE TAMIE J [US]; GOLDSTEIN JASON [US]; ANDE) 27 April 2006 (2006-04-27) cited in the application see claims 43-47 and Table 1	1-15

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2010	29/11/2010

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Merckling-Ruiz, V

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INTERNATIONAL SEARCH REPORT

International application No
PCT/AT2010/000303

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BENNER E.J. ET AL.: "Nitrated alpha-synuclein immunity accelerates degeneration of nigral dopaminergic neurons." PLOS ONE, vol. 3, no. 1, E1376, 2 January 2008 (2008-01-02), pages 1-20, XP002606274 see abstract, Table 4, Fig.10 and paragraph bridging pages 18-19	1-15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/AT2010/000303

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
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		AU 2003290548 A1	07-06-2004
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		CA 2584512 A1	27-04-2006
		EP 1809341 A2	25-07-2007
		JP 2008517928 T	29-05-2008