

ALPHA SYNUCLEIN THERAPEUTIC VACCINE

Field of the invention

The present invention relates to immunogenic compositions, and particularly vaccines, that can be employed for the prevention, alleviation or treatment of synucleinopathies. The immunogenic compositions can be employed for the prevention, alleviation or treatment of a condition (diseases, disorders and abnormalities) associated with alpha synuclein (referred to interchangeably herein as alpha-synuclein, α -synuclein, A-synuclein, aSynuclein, Alpha-Syn, A-syn, α -syn, aSyn or a-syn), specifically alpha synuclein aggregates including, but not limited to, 5
10 Lewy bodies and/or Lewy neurites, such as Parkinson's disease, Multiple System Atrophy, Lewy Body dementia (LBD; dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's disease dementia (PDD)), or Diffuse Lewy Body Disease.

Background of the invention

Many degenerative diseases are associated with extracellular or intracellular deposits of amyloid or amyloid-like proteins that contribute to the pathogenesis as well as to the progression of the 15
disease. The best characterized amyloid protein that forms extracellular aggregates is amyloid beta ($A\beta$).

Amyloid-like proteins that form mainly intracellular aggregates, include, but are not limited to alpha 20
synuclein, tau, TDP-43, and huntingtin (htt). Diseases involving alpha synuclein aggregates are generally listed as synucleinopathies (or alpha synucleinopathies) and these include, but are not limited to, Parkinson's disease (sporadic, familial with alpha synuclein mutations and/or copy number variations, familial with mutations other than alpha synuclein, pure autonomic failure and Lewy body dysphagia), Lewy Body dementia (LBD; dementia with Lewy bodies (DLB) ("pure" 25
Lewy body dementia), Parkinson's disease with dementia (PDD)), diffuse Lewy body disease (DLBD), Multiple System Atrophy (MSA), sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, and Down syndrome.

30 Alpha synuclein is a 140 amino acid long, cytosolic protein abundantly expressed in the CNS and localized in pre-synaptic terminals (Burré J., J Parkinsons Dis. 2015;5(4):699-713). Alpha synuclein is a natively unfolded protein but adopts secondary structure of mostly helical nature

upon association with lipid vesicles or membranes (Iwai *et al.*, Biochemistry 1995, 34(32), 10139-10145). The physiological function of alpha synuclein remains elusive.

The sequence of alpha synuclein can be divided into three main domains: 1) the N-terminal region comprising of residues 1-60, which contains 11-mer amphipathic imperfect repeat residues with highly conserved hexamer (KTKEGV; SEQ ID NO: 7). This region has been implicated in regulating alpha synuclein association to lipid membranes and its internalization; 2) the hydrophobic Non-Amyloid beta Component (NAC) domain spanning residues 61-95; which is essential for alpha synuclein fibrillization; and 3) the C-terminal region spanning residues 96-140 which is highly acidic and proline-rich, it has no distinct structural propensity.

Alpha synuclein has been shown to undergo several post translational modifications, including truncations, phosphorylation, ubiquitination, sumoylation, oxidation, nitration, acetylation, glycation, glycosylation, and/or transglutaminase covalent cross linking (Fujiwara *et al.*, Nat Cell Biol 2002, 4(2), 160-164; Hasegawa *et al.*, J Biol Chem 2002, 277(50), 49071-49076; Li *et al.*, Proc Natl Acad Sci U S A 2005, 102(6), 2162-2167; Oueslati *et al.*, Prog Brain Res 2010, 183, 115-145; Schmid *et al.*, J Biol Chem 2009, 284(19), 13128-13142; Dorval *et al.*, J Biol Chem. 2006, 281(15):9919-24; Ruzafa *et al.*, PlosOne 2017 12(5):e0178576; Ischiropoulos *et al.*, Ann N Y Acad Sci. 2003, 991, 93-100; Munch *et al.*, J Chem Neuroanat. 2000;20:253-257; Marotta *et al.*, Chembiochem. 2012;13:2665-2670).

Several phosphorylation sites have been detected in the carboxyl-terminal region on Tyr-125, -133, and -136, and on Ser-129 (Negro *et al.*, FASEB J 2002, 16(2), 210-212). Extensive and selective phosphorylation of alpha synuclein at Ser-129 is evident in synucleinopathy lesions, including Lewy bodies (Fujiwara *et al.*, Nat Cell Biol 2002, 4(2), 160-164). Other post-translational modifications in the carboxyl-terminal, including glycosylation on Ser-129 (McLean *et al.*, Neurosci Lett 2002, 323(3), 219-223) and nitration on Tyr-125, -133, and -136 (Takahashi *et al.*, Brain Res 2002, 938(1-2), 73-80), may affect aggregation of alpha synuclein. Truncation of the carboxyl-terminal region by proteolysis has been reported to play a role in alpha synuclein fibrillogenesis in various neurodegenerative diseases (Rochet *et al.*, Biochemistry 2000, 39(35), 10619-10626). Full-length as well as partially truncated and insoluble aggregates of alpha synuclein have been detected in highly purified Lewy bodies (Crowther *et al.*, FEBS Lett 1998, 436(3), 309-312).

Abnormal protein aggregation is a common feature in an aging brain and in several neurodegenerative diseases. In *in vitro* models, alpha synuclein readily assembles into filaments resembling those isolated from brain of patients with Lewy Body dementia and familial PD (Crowther *et al.*, FEBS Lett 1998, 436(3), 309-312). Alpha synuclein and its mutated forms (e.g. A53T and A30P) have a random coil conformation and do not form significant secondary structures in aqueous solution at low concentrations; however, at higher concentrations they are prone to self-aggregate, producing amyloid fibrils (Wood *et al.*, J Biol Chem 1999, 274(28), 19509-19512). Several differences in the aggregation behavior of the PD-linked mutants and the wild-type protein have been documented. Monomeric alpha synuclein aggregates *in vitro* to form stable fibrils via a metastable oligomeric (i.e., protofibril) state (Volles *et al.*, Biochemistry 2002, 41(14), 4595-4602).

Parkinson's disease (PD) is the most common neurodegenerative motor disorder. PD is mainly an idiopathic disease, although in at least 5% of the PD patients the pathology is linked to mutations in one or several specific genes. Several point mutations have been described in the alpha synuclein gene (A30P, E46K, H50Q, G51D, A53T) which cause familial PD with autosomal dominant inheritance. Furthermore, duplications and triplications of the alpha synuclein gene have been described in patients that developed PD underlining the role of alpha synuclein in PD pathogenesis (Lesage *et al.*, Hum. Mol. Genet., 2009, 18, R48-59). The pathogenesis of PD remains elusive, however, growing evidence suggests a role for the pathogenic folding and aggregation of the alpha synuclein protein that leads to the formation of amyloid-like fibrils. Indeed, the hallmarks of PD are the presence of intracellular alpha synuclein aggregate structures called Lewy Bodies in the nigral neurons, as well as the death of dopaminergic neurons in the substantia nigra and elsewhere. Alpha synuclein is a natively unfolded presynaptic protein that can misfold and aggregate into larger oligomeric and fibrillar forms which are linked to the pathogenesis of PD. Studies have implicated small soluble oligomeric and protofibrillar forms of alpha synuclein as the most neurotoxic species (Lashuel *et al.*, J. Mol. Biol., 2002, 322, 1089-102), however the precise role of alpha synuclein in neuronal cell toxicity remains to be clarified (review: Cookson, Annu. Rev. Biochem., 2005, 74, 29-52).

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Recent evidence from cellular and animal models suggests that pathological alpha synuclein, in particular aggregated alpha synuclein can spread from one neuron to another. Once inside the new cell alpha synuclein aggregates act as seeds, recruiting endogenous alpha synuclein and advancing protein aggregation (Luk *et al.*, Science. 2012, 338(6109):949-5; Tran *et al.*, Cell Rep.

2014, 7(6):2054-65). Moreover, the transsynaptic spreading of pathological, in particular aggregated alpha synuclein could explain the progressive advancing of Lewy pathology through defined anatomical connected brain areas in PD that was first described by Braak and colleagues (Braak *et al.*, *Neurobiol. Aging*. 2003; 24:197–211).

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The diagnosis of Parkinson's disease is largely clinical and depends on the presence of a specific set of symptoms and signs (the initial core feature being bradykinesia, rigidity, rest tremor and postural instability), a slowly progressive course, and a response to drug treatment. The final confirmation of the diagnosis is made by post-mortem neuropathological analysis.

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Besides Parkinson's disease, the accumulation of aggregated alpha-synuclein into Lewy bodies is a characteristic of all Lewy body diseases, including Parkinson's disease with dementia (PDD), and dementia with Lewy bodies (DLB) (Capouch *et al.*, *Neurol. Ther.* 2018, 7, 249-263). In DLB, Lewy Bodies are diffusely distributed throughout the cortices of the brain and in addition to Lewy
15 Bodies and neurites, more threads and dot-like structures (Lewy dots) were found to be immunopositive for alpha-synuclein phosphorylated at Ser-129 (Outeiro *et al.*, *Mol. Neurodegener.* 2019, 14, 5).

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Alpha-synuclein aggregates are also found in multiple system atrophy (MSA). MSA is a rare and sporadic neurodegenerative disorder that manifests with rapidly progressive autonomic and motor dysfunction, as well as variable cognitive decline. Such disorders include Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy. The disease can be clinically sub-classified in parkinsonian (MSA-P) or cerebellar (MSA-C) variant, depending on the predominant motor phenotype (Fanciulli *et al.*, *N. Engl. J. Med.* 2015; 372, 249–63). It is characterized by the
25 aggregation of alpha-synuclein in the cytoplasm of oligodendrocytes, forming glial cytoplasmic inclusions (GCIs). GCIs, consisting primarily of fibrillary forms of alpha-synuclein, are the neuropathological hallmark of MSA and are found throughout the neocortex, hippocampus, brainstem, spinal cord and dorsal root ganglia (Galvin *et al.*, *Arch Neurol.* 2001, 58,186-90). GCIs are considered a central player in the pathogenesis of MSA. A correlation between the GCI load
30 and the degree of neuronal loss has been reported in both the striatonigral and the olivopontocerebellar regions (Stefanova *et al.*, *Neuropathol. Appl. Neurobiol.* 2016, 42, 20-32). Furthermore, a causative link between GCIs and the induction of neuronal loss has been shown in transgenic mice overexpressing human alpha-synuclein in oligodendrocytes under various

oligodendroglia-specific promoters. A key event in the pathophysiological cascade is considered to be the permissive templating ('prion-like' propagation) of misfolded alpha-synuclein.

PD01A was developed for the treatment of synucleinopathies such as PD (Volc et al., Lancet Neurol 2020, 19: 591-600). PD01A is a peptide-KLH conjugate where the peptide moiety mimics the C-terminal region of human alpha synuclein (WO 2009/103105 A2). It targets alpha synuclein while avoiding closely related protein family members including β -Synuclein (bSyn), which may have neuroprotective properties (Vigneswara *et al.*, PLoS One 8 (2013), e61442). Increasing evidence points to a causal role of alpha synuclein oligomers and aggregates in the processes that lead to neurodegeneration in PD. Lewy bodies or Lewy neurites are the histopathological signature markers of PD; they appear mainly in neurons and are predominantly composed of misfolded, fibrillar alpha synuclein. Furthermore, several lines of evidence in animal models support the theory that reducing accumulations of oligomeric and/or aggregated alpha synuclein may have disease-modifying effects.

It has been shown that vaccination with PD01A resulted in the decreased accumulation of alpha synuclein oligomers and improved memory and motor defects in two mouse models of synucleopathies covering PD and MSA (multiple system atrophy) (Mandler *et al.*, Acta Neuropathol. 127 (2014), 861; Mandler *et al.*, Molecular Neurodegeneration 10 (2015), 10).

Vaccination with PD01 mimotopes targeting alpha synuclein, has proven efficacy in various animal models of alpha synuclein aggregation disorders, reducing alpha synuclein pathology, preservation of neuroinflammation, as well as amelioration of behavior deficits (Mandler *et al.* 2014; WO 2009/103105 A1, WO 2017/076873 A1,). These peptides turned out to be safe and well-tolerated vaccines which are able to induce target-specific antibodies in humans (Volc et al., Lancet Neurol 2020, 19: 591-600). PD01A was assessed in a Randomized, Placebo-controlled, Parallel Group, Patient-blind, Phase I Study Assessing the Safety and Exploring the Immunogenicity/Therapeutic Activity of AFFITOPE® PD01A and PD03A in Patients With Early Multiple System Atrophy (NCT02270489), where patients received 4 doses in 4-weekly intervals with a study duration of 52 weeks (Study AFF009). PD01 has been further assessed in a Randomized, Controlled, Parallel Group, Patient-blinded, Single-center, Phase I Pilot Study to Assess Tolerability and Safety of Repeated Subcutaneous Administration of Two Doses of AFFITOPE® PD01A Formulated With Adjuvant to Patients With Parkinson's Disease (NCT01568099) with patients receiving 4 injections of two different doses (Study AFF008). PD01

was assessed in an AFF008E: Observational Follow-up Extension Study to Evaluate Long-term Safety and Tolerability of Immunization With AFFITOPE® PD01A Applied During AFF008 in Patients With Parkinson's Disease (NCT01885494). PD01 was assessed in a Phase IB Follow-up, Randomized, Controlled, Parallel Group, Patient-blinded, Single-center Study to Assess One
5 Boost With AFFITOPE® PD01A With Regard to Safety/Tolerability, Immunological + Clinical Activity in Patients Who Have Received the Vaccine Within the Study AFF008 (NCT02216188). PD01A was also assessed in a Phase IB Follow-up, Controlled, Parallel Group, Single-center Study to Assess a Second Boost Immunization With AFFITOPE® PD01A With Regard to Safety/Tolerability, Immunological and Clinical Activity in Patients Who Have Participated in the
10 AFF008 Program (NCT02618941).

WO 2018/151821 A1 discloses antibodies to alpha synuclein which are useful for diagnosing, treating and preventing neurodegenerative diseases. These antibodies are created by immunizing with native alpha synuclein or the allelic variant A53T and should bind preferentially
15 to preformed fibrils ("PFF").

WO 2005/013889 A2 discloses fragments of native alpha synuclein which are useful for the treatment or prophylaxis of a LB disease (LBD) or to provide monoclonal antibodies for the treatment or prophylaxis of LBD.
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Despite significant clinical investments, there is currently no therapeutically active agent available with disease modifying properties in humans. At present, there is no vaccine available to prevent the early stages of Parkinson's Disease, emphasizing the urgent need for disease-modifying treatments. Current symptomatic treatments for PD primarily address motor symptoms through
25 the use of dopaminergic strategies, anticholinergic drugs, or deep brain stimulation, which are of symptomatic benefit only and are rife with side effects. Consequently, there is a need for the development of novel, disease altering treatment strategies.

Summary of the invention

It is an object of the present invention to provide immunogenic compositions that can be employed
30 to treat, alleviate and/or prevent a disease, disorder or abnormality associated with pathological alpha synuclein, in particular alpha synuclein aggregates, such as Parkinson's Disease.

The invention generally relates to immunogenic compositions, which when administered to a subject induce the production of antibodies that inhibit and/or delay the formation of pathological alpha synuclein, in particular alpha synuclein aggregation and/or prevent cell to cell spreading of pathological alpha synuclein, in particular aggregated alpha synuclein. It has been surprisingly
5 found that the immunogenic compositions of the invention induce the production of antibodies with a high degree of preference for alpha synuclein aggregates over alpha synuclein monomers.

Alpha synuclein is a soluble protein that has the propensity to spontaneously aggregate and form soluble oligomers or soluble/insoluble protofibrils or mature fibrils or detergent-insoluble
10 aggregates under certain conditions. Seeded alpha synuclein aggregation is the aggregation accelerated by (pre-existing) pathological alpha synuclein, so called "seeds". The cell-to-cell spreading of pathological and/or aggregated alpha synuclein renders immunotherapy as a compelling target for new therapeutic approaches aiming to alleviate, treat, retard or halt the progression of PD and other synucleinopathies. The immunogenic compositions described herein
15 induce antibodies that inhibit and/or delay seeding and/or spontaneous alpha synuclein aggregation and/or prevent cell to cell spreading of pathological alpha synuclein, in particular aggregated alpha synuclein. This functional feature allows the antibodies to bind to alpha synuclein seeds in the extracellular space to either neutralize the seeds and consequently delay or inhibit the propagation of pathological alpha synuclein, particularly alpha synuclein aggregates
20 or facilitate the clearance of these spreading species. The development of such therapies for PD and other synucleinopathies addresses an unmet medical need since currently only symptomatic treatments are available.

Thus, the invention relates to immunogenic compositions that, when administered to a subject,
25 induce antibodies which:

- (i) delay or inhibit the propagation of pathological alpha synuclein, particularly alpha synuclein aggregates; and/or
- (ii) are capable of recognizing and binding to pathological alpha synuclein, particularly aggregated alpha synuclein, *in vitro* and/or *in vivo*, with highly preferential binding to
30 alpha synuclein aggregates over alpha synuclein monomers.

Accordingly, the invention relates in its broadest aspect to immunogenic compositions which, when administered to a subject, induce antibodies which bind alpha synuclein. As demonstrated

herein, the immunogenic compositions induce antibodies that delay or inhibit the propagation of pathological alpha synuclein, particularly alpha synuclein aggregates. They prevent cell to cell spreading of alpha synuclein aggregates. As demonstrated herein, the immunogenic compositions induce antibodies that preferentially bind to aggregated alpha synuclein *in vitro* and/or *in vivo*, over alpha synuclein monomers. The level of selectivity for alpha synuclein aggregates over alpha synuclein monomers is surprising. As demonstrated herein, the immunogenic compositions induce antibodies that prevent cell to cell spreading of pathological alpha synuclein, in particular aggregated alpha synuclein.

- 10 Immunogenic compositions of the invention may, when administered to a subject, induce the production of antibodies that inhibit and/or delay aggregation of alpha synuclein protein or fragments thereof.

- 15 In one embodiment, immunogenic compositions of the invention induce antibodies that inhibit the formation of alpha synuclein aggregates, including but not limited to, Lewy Bodies, Lewy Neurites, and/or glial cytoplasmic inclusions.

- 20 In one embodiment, immunogenic compositions of the invention induce antibodies that selectively bind aggregated alpha synuclein and/or pathological alpha synuclein in preference to non-aggregated alpha synuclein and/or non-pathological alpha synuclein (particularly monomeric alpha synuclein). These induced antibodies demonstrate surprisingly higher selectivity for aggregated alpha synuclein and/or pathological alpha synuclein in preference to non-aggregated alpha synuclein and/or non-pathological alpha synuclein (particularly monomeric alpha synuclein).

- 25 The immunogenic compositions of the invention have been shown to generate a favourable antibody response in combination with safety, as demonstrated by administration to non-human primates (NHP, Example 3 herein). Repeated administration of the immunogenic compositions of the invention was safe and well tolerated in NHP and resulted in a substantial antibody response binding to the pathological species of alpha synuclein.
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The immunogenic compositions of the invention are safe and well-tolerated and induce production of target-specific antibodies when administered to humans.

In one aspect, the invention provides an immunogenic composition comprising a conjugate of an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1). The antigenic peptide comprises or consists of the amino acid sequence DQPVLDP (SEQ ID NO: 2). The immunogenic composition further comprises a carrier protein comprising a detoxified form of diphtheria toxin. The number of alpha synuclein antigenic peptides conjugated to each carrier protein is between 10 and 24 and preferably between 12 and 14.

In one aspect, the invention provides an immunogenic composition comprising a conjugate of an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1). The antigenic peptide comprises or consists of the amino acid sequence DQPVLDP (SEQ ID NO: 2). The immunogenic composition further comprises a carrier protein comprising a detoxified form of diphtheria toxin. The concentration ratio on a grams per unit volume basis of antigenic peptide to carrier protein is around 1:2 to 1:50, preferably around 1:3 to 1:6. The antigenic peptide may be in a concentration of around 20-200 µg/ml, preferably around 150 µg/ml relative to a carrier protein concentration of around 40-1200 µg/ml, preferably around 582-825 µg/ml.

In one aspect, the invention provides a unit dose immunogenic composition comprising a conjugate of an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1). The antigenic peptide comprises or consists of the amino acid sequence DQPVLDP (SEQ ID NO: 2). The immunogenic composition comprises a carrier protein comprising a detoxified form of diphtheria toxin. The antigenic peptide is in a dose of 10-100 µg, 15-75 µg or 16-75 µg, preferably 75 µg. This is the dose that is administered to the subject in a single administration. The corresponding dose of the carrier protein may be between around 290 and around 420 µg of carrier protein. The unit dose may be 0.5 ml.

In one aspect, the invention provides an immunogenic composition comprising a conjugate of an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1). The antigenic peptide comprises or consists of the amino acid sequence DQPVLDP (SEQ ID NO: 2). The immunogenic composition further comprises a carrier protein comprising a detoxified form of diphtheria toxin conjugated to the antigenic peptide via a linker, wherein the linker is a heterobifunctional linker comprising a NHS

ester and a maleimide reactive group, and preferably comprises N-γ-maleimidobutyl-oxysuccinimide ester (GMBS). The antigenic peptide may comprise a cysteine residue, preferably peptide bonded to the N terminal amino acid of the antigenic peptide, giving the amino acid sequence CDQPVLPD (SEQ ID NO: 5). The cysteine provides a thiol (sulfhydryl) group that can react with a maleimide to form a stable thioether linkage with the linker. Optionally, the linker consists of GMBS reacted with a N terminal cysteine in the antigenic peptide.

In one aspect, the invention provides an immunogenic composition comprising a conjugate of an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1) (amino acids 115-121). The antigenic peptide comprises or consists of the amino acid sequence DQPVLPD (SEQ ID NO: 2). The immunogenic composition comprises a carrier protein comprising a detoxified form of diphtheria toxin. The immunogenic composition is formulated for intramuscular administration, or administered intramuscularly, to a subject. Preferably, the immunogenic composition is administered to the deltoid muscle of the arm. Alternatively, the immunogenic composition may be administered to the anterolateral muscle of the thigh.

As used herein, an "immunogenic composition" refers to a composition that can elicit an immune response, specifically an antibody response, in a subject to whom the composition is administered. Given the immunogenic composition elicits an antibody response against pathological alpha synuclein, the immunogenic composition may also be described as a vaccine. As used herein, "subject" means any animal, preferably a mammal, with the capacity to generate an adaptive immune response (antibody response) and to whom the immunogenic compositions of the invention are administered. The term "mammal" as used herein, encompasses any mammal. Examples of mammals include, but are not limited to, cows, horses, sheep, pigs, cats, dogs, mice, rats, rabbits, guinea pigs, non-human primates (NHPs) such as monkeys or apes, humans, etc. Given the therapeutic application of the immunogenic compositions, the preferred subject is a human.

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As described herein, when administered to a subject, the immunogenic compositions of the invention induce the production of antibodies that preferentially bind to alpha synuclein aggregates over alpha synuclein monomers. The antibodies produced are polyclonal. The antibodies may also be referred to as "induced antibodies" herein. The terms "alpha synuclein

antibody", "anti-alpha synuclein antibody" and "an antibody that binds to pathological and/or aggregated alpha synuclein" or simply "antibody" as used herein refer to an antibody that is capable of binding pathological alpha synuclein, in particular aggregated alpha synuclein, including, but not limited to, Lewy bodies, Lewy Neurites or glial cytoplasmic inclusions. The antibodies bind with sufficient affinity and specificity such that they are useful as a therapeutic agent in targeting pathological alpha synuclein, in particular aggregated alpha synuclein.

Antibodies induced by administering the immunogenic compositions of the invention show no or only a significantly lower immune reactivity to beta-synuclein than to alpha synuclein. In contrast thereto, antibodies induced by immunising with the original alpha synuclein epitope comprising DMPVDPD bind to both the alpha synuclein and the beta-synuclein. Therefore, unlike the original alpha synuclein or fragment(s) thereof, the immunogenic compositions according to the present invention provide a specificity towards the disease related agent and avoids cross reactivity with disease unrelated beta-synuclein. 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.

Human alpha synuclein has the sequence of SEQ ID NO: 6
 (MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKE
 QVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQLGKNEEGAPQEGILEDMPVDPDNE
 AYEMPSEEGYQDYEPEA).

Alpha synuclein aggregates are multimeric assemblies of alpha synuclein monomers that can form either soluble oligomers or soluble/insoluble protofibrils or mature beta-sheet rich fibrils which coalesce into intracellular deposits detected as a range of Lewy pathologies in Parkinson's disease and other synucleinopathies. Alpha synuclein under physiological conditions does not adopt an ordered tertiary structure, rather it is classified as a natively unfolded protein which can exist as a mixture of dynamic and flexible structural conformations. Misfolded alpha synuclein can form multimeric intermediate oligomeric structures which eventually assemble into highly-ordered fibrillar aggregates. The term "aggregated alpha synuclein" as used herein, refers to insoluble or soluble oligomeric and/or polymeric structures composed of alpha synuclein misfolded monomers and/or multimers and/or assemblies of monomers.

Pathological alpha synuclein is misfolded or aggregated or post-translationally modified alpha synuclein that is the main component of Lewy pathologies; Lewy pathologies can be detected as having the following morphologies: Lewy bodies, Lewy neurites, premature Lewy bodies or
5 pale bodies, perikaryal deposits with diffuse, granular, punctate or pleomorphic patterns. Moreover, pathological alpha synuclein is the major component of intracellular fibrillary inclusions detected in oligodendrocytes also referred to as glial cytoplasmic inclusions and in neuronal somata, axons and nuclei (referred to as neuronal cytoplasmic inclusions) that are the histological hallmarks of multiple system atrophy. Pathological alpha synuclein in Lewy
10 pathologies often displays substantial increase in post-translational modifications such as phosphorylation, ubiquitination, nitration, and truncation.

Seeds are multimeric beta-sheet rich structures which are composed of alpha synuclein, and may also comprise (i.e. in addition to alpha synuclein) other amyloidogenic proteins (e.g. Tau,
15 Amyloid β), which can accelerate the aggregation kinetics of alpha synuclein by elongating the growing multimer and/or by acting as templates for the nucleation of monomers on the seed surface.

Spontaneous aggregation of alpha synuclein is the aggregation process that progresses without
20 the addition of seeds. Alpha synuclein is a soluble protein that has the propensity to spontaneously aggregate and form soluble oligomers or soluble/insoluble protofibrils or mature fibrils or detergent-insoluble aggregates under certain conditions.

Seeding is the process in which seeds induce the aggregation of the native alpha synuclein
25 protein.

Lewy bodies are abnormal aggregates of protein that develop inside nerve cells in Parkinson's disease (PD), Lewy body dementia and other synucleinopathies. Lewy bodies appear as spherical masses that displace other cell components. Morphologically, Lewy bodies can be classified as
30 being brainstem or cortical type. Classic brainstem Lewy bodies are eosinophilic cytoplasmic inclusions consisting of a dense core surrounded by a halo of 5-10-nm-wide radiating fibrils, the primary structural component of which is alpha synuclein; cortical Lewy bodies differ by lacking a halo. The presence of Lewy bodies is a hallmark of Parkinson's disease.

Lewy neurites are abnormal neuronal processes in diseased neurons, containing granular material, abnormal alpha synuclein filaments similar to those found in Lewy bodies, dot-like, varicose structures and axonal spheroids. Like Lewy bodies, Lewy neurites are a feature of alpha synucleinopathies such as dementia with Lewy bodies, Parkinson's disease, and multiple system atrophy.

Glial cytoplasmic inclusions (also referred to as Papp-Lantos inclusions) consist of insoluble alpha synuclein filamentous aggregates detected in oligodendrocytes in the white matter of multiple system atrophy brains. Alpha synuclein aggregates in neuronal somata, axons and nuclei, referred to as neuronal cytoplasmic inclusions, are characteristic cytopathological features of multiple system atrophy. The detection of glial cytoplasmic inclusions is considered a hallmark for the neuropathological diagnosis of multiple system atrophy.

The term "bind to" or "binding to" as used in the context of the present invention defines a binding (interaction) of at least two "antigen-interaction-sites" with each other. The term "antigen-interaction-site" defines, in accordance with the present invention, a motif of a polypeptide, i.e., a part of the antibody, which shows the capacity of specific interaction with a specific antigen or a specific group of antigens of alpha synuclein. The target epitope within alpha synuclein is the amino acid sequence DMPVDPD (SEQ ID NO: 1).

As already introduced, the antibodies produced when the immunogenic compositions of the invention are administered to a subject show surprisingly high levels of preference for binding to aggregated alpha synuclein over alpha synuclein monomers. Moreover, this advantageous recognition profile is maintained over time (i.e. does not significantly diminish). This preference can be demonstrated by any suitable method in the art. It may be demonstrated in a sample taken from the subject to whom the immunogenic composition has been administered. A preferred sample type is serum. Alternatively, the preference may be demonstrated using purified antibody preparations. Any suitable purification technique may be employed as would be understood by one skilled in the art. As exemplified herein, preferential binding may be demonstrated through an immunoassay, such as a competitive inhibition assay. One specific example of a suitable assay is a competitive Meso Scale Discovery (MSD) assay. Reference is made to Example 3 herein for details of the assay. Based on such assays, the concentration needed to inhibit half of the measured response, the IC50, can be determined at a given timepoint. The IC50 values can

also be used to derive relative preference of the antibodies to bind alpha synuclein aggregates over alpha synuclein monomers.

The immunogenic compositions when administered to a subject induce the production of antibodies with an IC50 for alpha synuclein aggregates on a single nanomolar scale. On the other hand, the immunogenic compositions when administered to a subject induce the production of antibodies with an IC50 for alpha synuclein monomers on a thousand nanomolar scale. Thus, the preference for aggregates over monomers may be over three orders of magnitude (over 1000-fold, or 3-log). The immunogenic compositions of the invention may induce the production of antibodies that have an IC50 for alpha synuclein aggregates of around 5 nM or less, around 4.5 nM or less, around 4.27 nM or less, around 4 nM or less, around 3.5 nM or less, around 3 nM or less, around 2.99 nM or less, around 2.98 nM or less, around 2.5 nM or less, around 2.4 nM or less, around 2 nM or less, around 1.5 nM or less, around 1.41 nM or less, around 1.35 nM or less, around 1.24 nM or less, around 1.131 nM or less, around 1 nM or less, around 0.5 nM or less or around 0.29 nM or less. Advantageously, such values may be determined by competitive Meso Scale Discovery (MSD) assay. Details of a suitable assay are found in Example 3.

The immunogenic compositions of the invention may, on the other hand, induce the production of antibodies that have an IC50 for alpha synuclein monomers of around 2500 nM or more, around 3000 nM or more, or around 3457 nM or more. Advantageously, such values may be determined by competitive Meso Scale Discovery (MSD) assay (Example 3).

The immunogenic composition thus may induce the production of antibodies wherein the antibodies have:

- a. an IC50 for alpha synuclein aggregates of around 5 nM or less, around 4.5 nM or less, around 4.27 nM or less, around 4 nM or less, around 3.5 nM or less, around 3 nM or less, around 2.99 nM or less, around 2.98 nM or less, around 2.5 nM or less, around 2.4 nM or less, around 2 nM or less, around 1.5 nM or less, around 1.41 nM or less, around 1.35 nM or less, around 1.24 nM or less, around 1.131 nM or less, around 1 nM or less, around 0.5 nM or less or around 0.29 nM or less, for example as measured by competitive Meso Scale Discovery (MSD) assay; and

- b. an IC50 for alpha synuclein monomers of around 2500 nM or more, around 3000 nM or more, or around 3457 nM or more, for example as measured by competitive Meso Scale Discovery (MSD) assay.
- 5 The immunogenic compositions may induce the production of antibodies wherein the antibodies have an IC50 for alpha synuclein monomers that is at least 500-fold, at least 1000-fold, at least 2500-fold, at least 5000-fold, at least 7500-fold, at least 10000-fold or at least 11920-fold, greater than the IC50 for alpha synuclein aggregates.
- 10 As already introduced, the antibodies produced when the immunogenic compositions of the invention are administered to a subject delay or inhibit the propagation of pathological alpha synuclein, particularly alpha synuclein aggregates. This ability can be demonstrated by any suitable method in the art. It may be demonstrated in a sample taken from the subject to whom the immunogenic composition has been administered. A preferred sample type is serum.
- 15 Alternatively, the preference may be demonstrated using purified antibody preparations. Any suitable purification technique may be employed as would be understood by one skilled in the art. As exemplified herein, the delay or inhibition of propagation of pathological alpha synuclein, particularly alpha synuclein aggregates may be measured using a primary neuronal seeding assay, such as a rat primary neuronal seeding assay. The assay, in particular a primary
- 20 neuronal seeding assay, may be used to measure the reduction in number of intracellular alpha synuclein aggregates when compared to a control lacking antibodies (such as serum from a subject to which the immunogenic composition has not been administered). The assay may use serum from a subject treated with one or more doses of an immunogenic composition according to the invention. Various serum dilutions may be utilised, such as 1/500, 1/200 and/or 1/100. A
- 25 suitable primary neuronal seeding assay is disclosed in Example 2, which shows the reduction of alpha synuclein pS129-positive inclusions in the presence of ACI-7104.056 immunized sera (diluted at 1/500, 1/200 and 1/100) compared to non-immunized serum.

Thus, the immunogenic composition may induce the production of antibodies that reduce the

30 number of intracellular alpha synuclein aggregates by at least 10% , by at least 15% or by at least 25% as compared to a control lacking the antibodies. The reduction may be measured by a neuronal seeding assay. The immunogenic composition may induce the production of antibodies that reduce the number of intracellular alpha synuclein aggregates at least 10% when measured in serum at a 1/500 dilution, 15% when measured in serum at a 1/200 dilution

or 25% when measured in serum at a 1/100 dilution as compared to a control lacking the antibodies. The reduction may be measured by a neuronal seeding assay.

The antigenic peptide is a mimotope of the 7 amino acids of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1). The mimotope does not necessarily need to be of the same length as the original epitope from alpha synuclein provided that it generates at least an equivalent antibody response when included in an immunogenic composition of the invention. The core sequence of the mimotope is DQPVLDP (SEQ ID NO: 2) and thus contains two amino acid changes compared to the native sequence and is of 7 amino acids in length. In some embodiments, the antigenic peptide is 7 to 30, 7 to 20, 7 to 16, 8 or 7 amino acid residues in length. Preferably, the antigenic peptide is at least 7 amino acids, and preferred lengths may be up to 14, 16 or 20 amino acids residues (e.g. 7 or 8 to 20, 7 or 8 to 16 etc.). The antigenic peptide may be at least 50%, 55%, 60%, 65%, 70% or 75% identical to the amino acid sequence of SEQ ID NO: 1. The antigenic peptide may be at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85% or 87.5% identical to the amino acid sequence of SEQ ID NO: 2.

The antigenic peptide preferably consists of the amino acid sequence DQPVLDP (SEQ ID NO: 2). In other embodiments, the antigenic peptide consists of the amino acid sequence DQPVLDPN (SEQ ID NO: 3) or DQPVLDPG (SEQ ID NO: 4).

The immunogenic compositions of the invention comprise a conjugate of an antigenic peptide and a carrier protein. The carrier protein comprises a detoxified form of diphtheria toxin and is preferably a genetically detoxified form. The preferred detoxified form of diphtheria toxin included in the immunogenic compositions of the invention is CRM197. CRM197 is a genetically detoxified mutant of diphtheria toxin (DT) that carries a substitution mutation at amino acid position 52 of glutamic acid for glycine (G52E), which results in a loss of ADP-ribosyltransferase activity.

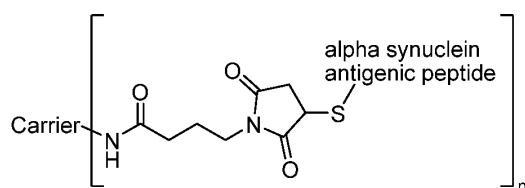
Generally, the antigenic peptide is conjugated with the carrier protein via a linker. This permits reliable directed conjugation of the antigenic peptide with the carrier protein, resulting in controlled levels of antigenic peptide and carrier protein in the immunogenic composition. In one embodiment, a cysteine residue is incorporated into the antigenic peptide to facilitate reaction with the linker. This may be peptide bonded to the antigenic peptide, although it is present to facilitate linker attachment and thus is not considered to be the antigenic part of the peptide.

Cysteine provides a thiol (sulfhydryl) group that can react with a maleimide to form a stable thioether linkage. In one embodiment, the cysteine residue is attached, typically via a peptide bond, to the N-terminus and/or C-terminus of the antigenic peptide. Preferably, the cysteine is attached to the N-terminus of the antigenic peptide to facilitate a reaction with the linker. Thus, the antigenic peptide may comprise or consist of the amino acid sequence CDQPVLPD (SEQ ID NO: 5).

In a further embodiment, the linker comprises a chemical linker. The linker may be a heterobifunctional compound, of which several examples are known in the art; see for example Bioconjugate Techniques, Greg T. Hermanson. The linker may comprise NHS-ester and maleimide reactive groups. The linker may be a heterobifunctional linker, preferably comprising a NHS ester and a maleimide reactive group. Thus, the maleimide may react with a thiol (sulfhydryl) group in the cysteine attached to the antigenic peptide to form a stable thioether linkage. The NHS ester may react with amine groups in the carrier protein, in particular through lysine residues in the carrier protein. Preferably, the linker comprises N-γ-maleimidobutyl-oxysuccinimide ester (GMBS, CAS No.: 80307-12-6).

In the immunogenic compositions of the invention, the number of alpha synuclein antigenic peptides conjugated to each carrier protein is at least 10, and generally between 10 and 24. Although the range may be between 10 and 24, the mean, or average, number of alpha synuclein antigenic peptides conjugated to each carrier protein is preferably between 12 and 14. Immunogenic compositions of the invention are optimised to produce beneficial antibody responses when administered to subjects, as demonstrated herein.

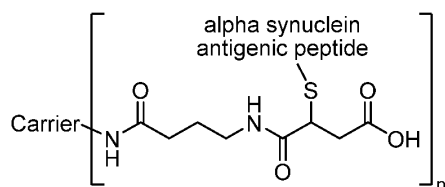
In one embodiment, the conjugate has the structure of Formula (I):



wherein n is an integer of 10 to 24

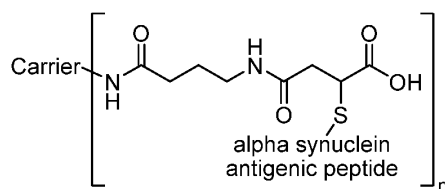
Formula (I) shows the carrier protein (carrier) conjugated with 10-24 alpha synuclein antigenic peptides via a GMBS linker. The maleimide hydrolysis products, which may form in the

presence of water, are shown in Formula (II) and (III) below. Thus, in another embodiment, the conjugate has the structure of Formula (II):



wherein n is an integer of 10 to 24.

- 5 In an additional embodiment, the conjugate has the structure of Formula (III):



wherein n is an integer of 10 to 24.

- In further embodiments, the immunogenic composition comprises a mixture of conjugates with formulas I (closed ring structure) and II (open ring structure, hydrolysed), formulas I and III, formulas II and III, or all of formulas I, II and III. The relative proportions of the different forms may depend on the conditions of storage.

- In the immunogenic compositions of the invention, the antigenic peptide may be in a concentration of around 100-200 $\mu\text{g/ml}$ relative to a carrier protein concentration of around 400-1000 $\mu\text{g/ml}$. The immunogenic composition of the invention may have a concentration ratio on a grams per unit volume basis of antigenic peptide to carrier protein is around 1:2 to 1:50, preferably around 1:3 to 1:6. Such immunogenic compositions provide for highly beneficial antibody responses when administered to a subject, as described herein. In preferred embodiments, the antigenic peptide is present in a concentration of around 20-200 $\mu\text{g/ml}$, preferably 150 $\mu\text{g/ml}$. In preferred embodiments, the carrier protein is present in a concentration of around 40-1200 $\mu\text{g/ml}$, 550-850 $\mu\text{g/ml}$ or 580-830 $\mu\text{g/ml}$, preferably 582-825 $\mu\text{g/ml}$. Thus, the antigenic peptide may be present in a concentration of around 20-200 $\mu\text{g/ml}$, preferably 150 $\mu\text{g/ml}$ relative to the carrier protein concentration of around 40-1200 $\mu\text{g/ml}$, 550-850 $\mu\text{g/ml}$ or 580-830 $\mu\text{g/ml}$, preferably 582-825 $\mu\text{g/ml}$.

Immunogenic compositions of the invention can have defined amounts of antigenic peptide and carrier proteins. In addition, the immunogenic compositions can also be in defined volumes and optionally have additional components according to the invention as defined herein, for example the presence of adjuvants. The immunogenic composition may comprise between around 16
5 and around 75 µg antigenic peptide, preferably 75 µg antigenic peptide. The immunogenic composition may comprise between around 290 and around 420 µg of carrier protein. The immunogenic composition is typically in liquid form, for ease of administration. The immunogenic composition may be in any suitable volume, typically in the range of around 0.1-1ml and is preferably in a volume of 0.5 ml. The immunogenic composition of the invention may
10 comprise 75 µg antigenic peptide and between around 290 and around 420 µg of carrier protein in a volume of 0.5 ml. Optionally, the immunogenic composition of the example further contains 0.5 mg aluminium hydroxide adjuvant.

As presented herein, the concentration of the antigenic peptide is expressed with reference to a
15 peptide incorporating an N-terminal cysteine. Therefore, the concentration of the antigenic peptide is expressed with reference to CDQPVLPD (SEQ ID NO: 5).

In some embodiments, the immunogenic composition further comprises an adjuvant. In some embodiments, the adjuvant is present in a concentration of 1 mg/ml. In preferred embodiments,
20 the immunogenic composition comprises 0.5 mg adjuvant. Preferably the adjuvant is an aluminium containing adjuvant. In one embodiment, the adjuvant is aluminium hydroxide. The aluminium hydroxide may be hydrated, for example in the form of a hydrogel. An example of a suitable adjuvant is European Pharmacopoeial grade (Aluminium-oxyhydroxide, monograph 1664), more specifically to the product manufactured by Brenntag Biosector (2% Alhydrogel)
25 tested towards EP compliance. Alhydrogel is available in three varieties: Alhydrogel 1.3%; Alhydrogel 2% and Alhydrogel "85". Alhydrogel 2% was elected as the International Standard Preparation for aluminium hydroxide gels. The preparation may be aseptically formulated into a suitable buffer, preferably an isotonic phosphate buffer (1mM to 100 mM), preferably at a concentration of ≥ 1.0 mg/ml Alhydrogel (given as Al_2O_3 equivalent; this metric (Al as " Al_2O_3
30 equivalent") is used generally herein; accordingly, all doses and amounts referred to herein, as far they relate to aluminium oxyhydroxide refer to Al_2O_3 equivalents (of aluminium oxyhydroxide (Alhydrogel)), even more preferably at a concentration of ≥ 1.5 mg/ml Alhydrogel (given as Al_2O_3 equivalent), most preferably at a concentration of ≥ 2.0 mg/ml Alhydrogel (given as Al_2O_3 equivalent). The amount of aluminium salt for Alhydrogel is given as Al_2O_3 equivalent in line with

the strength as stated by the manufacturer (i.e. 2% Alhydrogel equates to 2% Al_2O_3 , i.e. 20 mg/mL). This concentration is directly convertible into the respective concentration of aluminium by using the respective molecular masses (20 mg/mL Al_2O_3 (Mw 101,96) corresponds to 10.6 mg/mL aluminium (molecular mass 26,98)).

5

The immunogenic compositions of the invention are formulated for administration to a subject. They may, therefore, be specified as pharmaceutical compositions. They may comprise any pharmaceutically acceptable excipient. The immunogenic compositions of the inventions generally include one or more buffers. Therefore, the immunogenic composition may further

10 comprise one or more, and preferably all, of NaCl, KH_2PO_4 , Na_2HPO_4 and KCl. The Na_2HPO_4 is preferably a dodecahydrate. In one embodiment, the NaCl is present in a concentration of around 5-10 mg/ml, the KH_2PO_4 is present in a concentration of around 0.1-0.5 mg/ml, the Na_2HPO_4 is present in a concentration of around 2-4 mg/ml, and the KCl is present in a concentration of around 0.1-0.5 mg/ml.

15

The immunogenic compositions of the invention may be prepared with different final amounts of antigenic peptide depending on how it is intended to be stored and the intended dose of administration. In one embodiment, the antigenic peptide is contained in an amount from 0.1 ng to 10 mg, preferably 10 ng to 1 mg, in particular 100 ng to 100 μg . Alternatively the antigenic

20 peptide is contained in an amount 100 fmol to 10 pmol, preferably 10 pmol to 1 pmol, in particular 100 pmol to 100 nmol.

25

As already stated, the immunogenic composition may be described as a vaccine. Vaccines are therapeutic and/or prophylactic compositions used in the prevention, alleviation or treatment of

conditions. According to the present invention, the vaccine may be used prophylactically and/or therapeutically. In the context of the invention the term vaccine may be used interchangeably with the term "active immunotherapy".

30

The immunogenic compositions of the invention may be administered, or formulated for administration, by any suitable route to the subject. Therefore, the immunogenic compositions may be administered, or formulated for administration, to a subject by one or more routes including subcutaneous, intravenous, intradermal or intramuscular administration. In a preferred embodiment, the immunogenic composition is administered, or formulated for administration, to a subject intramuscularly. The muscle that the immunogenic composition is administered to, or

is formulated for administration, may be the deltoid muscle of the arm or the anterolateral muscle of the thigh. Preferably, the muscle is the deltoid muscle of the arm.

In one aspect, the invention provides a ready to use vial containing 0.5-1 ml, preferably 0.75 ml, of an immunogenic composition of the invention. In one embodiment, the ready to use vial contains 0.75 ml of immunogenic composition, wherein the immunogenic composition comprises 112.5 µg antigenic peptide and 436.5-618.75 µg carrier protein, optionally further comprising 0.75 mg adjuvant. By "ready to use" means that the immunogenic composition is in a form suitable for direct administration to the subject. Suitable formulations are described herein.

In one aspect, the immunogenic composition according to the invention is for use in the prevention, alleviation or treatment of a condition associated with alpha synuclein, particularly with pathological alpha synuclein, more particularly with alpha synuclein aggregates. Such medical uses can be expressed in any form, including as methods of treatment of the subject. All embodiments herein apply to such methods or medical uses, however expressed. Thus, in one aspect, the use of an immunogenic composition according to the invention is provided for the manufacture of a medicament preventing, alleviating or treating a condition associated with alpha synuclein, particularly with pathological alpha synuclein, more particularly with alpha synuclein aggregates. Similarly, in one aspect, provided herein are methods of treatment of a condition associated with alpha synuclein, particularly with alpha synuclein aggregates. The methods may comprise administering an effective concentration or an effective amount of an immunogenic compositions described herein to a subject in need thereof. In some embodiments the immunogenic composition is administered for the prevention, alleviation of symptoms associated with, or treatment of a synucleinopathy, wherein an effective amount of the immunogenic composition comprising the antigenic peptide is administered to a patient in need thereof.

As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed either for prophylaxis or during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, preventing occurrence or recurrence of disease or disorder or abnormality, alleviation of symptoms, diminishment of any direct or indirect pathological consequences of the disease, decreasing the rate of disease progression, amelioration or palliation of the disease state, and remission or improved prognosis. In some embodiments, immunogenic compositions of the invention are used to delay development of a

disease or to slow the progression of a disease, disorder or abnormality. Treatment may relate to preventing, slowing down, halting, retaining and/or improving the motor capabilities or motor deficits, cognitive capabilities or cognitive deficits, or behavioral impairments of a subject suffering from a synucleinopathy. Treatment may relate to improving motor capabilities, in particular facial expression, speech, ocular motor dysfunction, tremor at rest, action tremor, increased tone, rapid alternating movement of hands, finger tapping, leg agility, Heel-Shin test, arising from chair, posture, body sway and/or gait; improving cognitive deficits, in particular as measured by MoCA (Montreal Cognitive Assessment) or Addenbrookes Cognitive Examination; and/or improving behavioral impairments, in particular using NPI scale, wherein the synucleinopathy is multiple system atrophy (MSA).

As discussed, the immunogenic compositions according to the invention are to for use in preventing, alleviating or treating a condition in a subject that is associated with alpha synuclein, particularly with pathological alpha synuclein, more particularly with alpha synuclein aggregates. Preferably, the condition is a synucleinopathy. 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 protein in selected populations of neurons and glia cells. Alpha synuclein 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 synucleinopathy may be selected from the group consisting of Parkinson's disease (PD) (sporadic, familial with alpha synuclein mutations or copy number variations, familial with mutations other than alpha synuclein, pure autonomic failure and Lewy body dysphagia), Lewy Body dementia (LBD; dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's disease dementia (PDD)), Diffuse Lewy Body Disease (DLBD), sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, multiple system atrophy (MSA) (Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy), inclusion-body myositis, traumatic brain injury, chronic traumatic encephalopathy, dementia pugilistica, tauopathies (Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, Frontotemporal dementia with Parkinsonism linked to chromosome 17 and Niemann-Pick type

C1 disease), Down syndrome, Creutzfeldt-Jakob disease, Huntington's disease, motor neuron disease, amyotrophic lateral sclerosis (sporadic, familial and ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (Hallervorden-Spatz syndrome), prion diseases, Gerstmann-Straussler-Scheinker disease, ataxia

5 telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gaucher disease, Krabbe disease as well as other lysosomal storage disorders (including Kufor-Rakeb syndrome and Sanfilippo syndrome), or rapid eye movement (REM) sleep behavior disorder. Preferably, the synucleinopathy is multiple system atrophy (MSA), dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), or Parkinson's disease (PD), more preferably early stage PD, more

10 preferably early stage idiopathic Parkinson's Disease, in particular as defined using modified Movement Disorder Society Criteria. As defined herein, early stage idiopathic Parkinson's Disease is determined by the modified Movement Disorder Society criteria as defined in Berg *et al* 2018 (incorporated by reference), after excluding any other known or suspected cause of Parkinson's Disease. Preferably, subject with early stage idiopathic Parkinson's Disease are

15 between 40 and 75 years old. In such subjects, motor symptoms may have been present for less than 2 years at the onset of treatment.

In a further embodiment, when the synucleinopathy is Parkinson's disease, Lewy Body dementia (LBD; dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's

20 disease dementia (PDD)), or Diffuse Lewy Body Disease, the immunogenic compositions provided herein are for: (i) improving motor capabilities, in particular activities of daily living (speech, salivation, swallowing, handwriting, cutting food and handling utensils, dressing, hygiene, turning in bed and adjusting bed clothes, falling, freezing when walking, walking, tremor, sensory complaints related to Parkinsonism), motor examination (speech, facial

25 expression, tremor at rest, action or postural tremor of hands, rigidity, finger taps, hand movements, rapid alternating movements of hands, leg agility, arising from chair, posture, gait, postural stability, body bradykinesia and hypokinesia, dyskinesias, clinical fluctuations), symptomatic orthostasis, repeated falls and syncope, and/or transient unexplained loss of consciousness; and/or (ii) improving cognitive deficits; and/or (iii) improving behavioral

30 impairments, in particular behavior and mood (intellectual Impairment, thought disorder, depression, motivation/initiative), delusions, hallucinations, agitation/aggression, depression/dysphoria, anxiety, elation/euphoria, apathy/indifference, irritability/lability, motor disturbance, nighttime behavior, and/or appetite/eating, deficits of attention, executive functions,

visuospatial ability, visual hallucination; and/or (iv) improving rapid eye movement (REM) sleep disorders, in particular insomnia, hypersomnolence.

Exemplary diseases or disorders or abnormality that may be prevented, alleviated or treated using an immunogenic composition of the invention include diseases, disorders or abnormalities associated with alpha synuclein aggregates including, but not limited, Lewy bodies, Lewy neurites and/or Glial cytoplasmic inclusions, that are manifested in a cognitive deficit or behavioral impairment, or motor deficit or impairment such as bradykinesia, rigidity, resting tremor or postural instability.

As discussed, the immunogenic compositions of the invention induce antibodies with advantageous binding properties selective for aggregated alpha synuclein over monomeric alpha synuclein and which can prevent seeding. Without wishing to be bound by theory, the therapeutic consequence of targeting aggregated alpha synuclein is to neutralise the toxic effects of these species and ameliorate or reverse phenotypes in subjects with the targeted conditions, including preventing seeding.

The immunogenic compositions of the invention are safe and well-tolerated and induce production of target-specific antibodies when administered to humans.

In one embodiment, the immunogenic compositions of the invention induce an anti-alpha-synuclein immunogenic response following two administrations of the immunogenic composition.

In one embodiment, the anti-alpha-synuclein immunogenic response is boosted as measured at least 2 weeks after the administration of a second dose of the immunogenic composition. In a preferred embodiment the anti-alpha-synuclein immunogenic response is increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150% or more as measured at least 2 weeks after the second administration of the immunogenic composition. For the avoidance of doubt, the increase is determined by comparing the anti-alpha-synuclein immunogenic response measured at least 2 weeks after the second administration of the immunogenic composition with the anti-alpha-synuclein immunogenic response measured at (to encompass shortly before) the time the second dose is administered.

In one embodiment, the anti-alpha-synuclein immunogenic response is boosted as measured at least 2 weeks after the administration of a third dose of the immunogenic composition. In a preferred embodiment the anti-alpha-synuclein immunogenic response is increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150% or more as measured at least 2 weeks after the third administration of the immunogenic composition. In one embodiment, the anti-alpha-synuclein immunogenic response is boosted as measured at least 2 weeks after the administration of a fourth dose of the immunogenic composition. In a preferred embodiment the anti-alpha-synuclein immunogenic response is increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150% or more as measured at least 2 weeks after the fourth administration of the immunogenic composition. For the avoidance of doubt, the increase is determined by comparing the anti-alpha-synuclein immunogenic response measured at least 2 weeks after the third administration of the immunogenic composition with the anti-alpha-synuclein immunogenic response measured at (to encompass shortly before) the time the third dose is administered. As used herein, "boosted" in the context of an antibody response refers to the antibody response that is maintained or enhanced after a subsequent administration as measured at least two weeks after the administration of the subsequent administration. For example, an antibody response is "boosted" by a subsequent administration, if there is an increase of the antibody titer when measured 2 weeks after the subsequent administration as compared with the antibody titer at or shortly before the subsequent administration.

The increase in anti-alpha-synuclein immunogenic response is measured by any appropriate means as described herein. The increase in immunogenic response may be measured by determining an increase in anti-aSyn peptide IgG titers (AU/mL), as exemplified in Example 4.

In one embodiment, treatment with the immunogenic composition prevents and/or slows down and/or halts and/or retains and/or improves the motor capabilities or motor deficits, cognitive capabilities or cognitive deficits, or behavioral impairments in a subject during and/or following treatment with the immunogenic composition.

In one embodiment, treatment with the immunogenic composition retains motor capabilities or improves motor deficits of a subject suffering from a synucleinopathy, including reducing bradykinesia, rigidity, resting tremor or postural instability.

In some embodiments, a method of retaining or increasing cognitive capacity of a subject suffering from a synucleinopathy is provided, comprising administering an immunogenic composition of the invention, to a subject in need thereof.

5

In one embodiment, treatment with the immunogenic composition improves clinical, cognitive or quality of life in a subject during and/or following treatment with the immunogenic composition as measured by one or more cognitive and clinical assessments selected from Montreal Cognitive Assessment (MoCA), Parkinson's Disease-Cognitive Rating Scale (PD-CRS);
 10 Movement Disorder Society-Nonmotor Rating Scale (MDS-NMS), Patient Global Impression Scale –Change (PGI-C), Patient Global Impression Scale –Severity (PGI-S), 39-Item Parkinson's Disease Questionnaire (PDQ-39), Starkstein Apathy Scale, Clinical Global Impression of Change (CGI-C), Clinical Global Impression of Severity (CGI-S) and Hospital Anxiety and Depression Scale (HADS).

15

The MoCA (Nasreddine *et al* 2005) is a brief, 30-question test that helps healthcare professionals detect cognitive impairments very early on, allowing for faster diagnosis and patient care. MoCA is the most sensitive test available for detecting Alzheimer's disease, measuring executive functions, and multiple cognitive domains which are important components
 20 not measured by the Mini-Mental State Examination (MMSE). The MoCA is a weighted questionnaire that covers domains of: visuospatial/executive function, naming, memory, attention, language, abstract thinking, delayed recall, memory index score, and orientation. Scoring is by drawing, multiple choices, pictorial, analogy, open-ended question, counting. Global scores range from 0 to 30, where 18 to 25 indicates mild cognitive impairment, 10 to 17
 25 indicates moderate cognitive impairment, and less than 10 indicates severe cognitive impairment. The memory index score (Kaur *et al* 2018) ranges from 0 to 15 (for version 8.1 only).

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The PD-CRS is a cognitive scale specifically designed to capture the full spectrum of cognitive defects associated with PD. It is a valid screening tool for mild cognitive impairment (MCI) in PD and is recommended for diagnosing PD-MCI-Level I (Pagonabarraga *et al* 2008; Santangelo *et al* 2017). This screening battery includes subtests to assess both cortical and subcortical functions and is composed by a total of 9 tasks explicitly designed for a brief and separate scoring of frontal subcortical tasks (sustained attention, working memory, alternating and action

verbal fluency, clock drawing, and immediate and delayed free recall verbal memory) and posterior cortical tasks (confrontation naming and clock copying).

The MDS-NMS (Chaudhuri *et al* 2020) is a revision of the Nonmotor Symptoms Scale (NMSS) developed to improve and refine the assessment of nonmotor symptoms in PD. This rater-completed assessment measures frequency and severity of 13 nonmotor domains: depression (5 items), anxiety (4 items), apathy (3 items), psychosis (4 items), impulse control and related disorders (4 items), cognition (6 items), orthostatic hypotension (2 items), urinary (3 items), sexual (2 items), gastrointestinal (4 items), sleep and wakefulness (6 items), pain (4 items), and other (5 items). The scale also has an additional Nonmotor Fluctuations subscale (8 items). Items are scored for frequency (from 0 [never] to 4 [majority of time]) and severity (from 0 [not present] to 4 [severe]), which are multiplied to generate the item total score. Scores for each domain and the total rating scale (maximum, 832 points) are calculated by summing the corresponding items. This questionnaire is scored by domains on a 5-point Likert/Likert-type Scale ranging from 0 to 4 (each item is scored twice), and with dichotomous response options. Global score ranges from 0 to 832, with a higher score indicating higher burden of nonmotor symptoms.

The Patient Global Impression scale (Guy 1976), also known as PGI, is the patient-reported outcomes counterpart to the CGI, which was published in 1976 by the US National Institute of Mental Health. It consists of 1 item based on the CGI and adapted to the patient. It mainly measures change in clinical status (PGI-C) but can also measure disease severity (PGI-S). The PGI-C based on the CGI-C developed by the National Institute of Health is scored on a 7-point Likert Scale: 1, "very much improved," 2, "much improved," 3, "minimally improved," 4, "no change," 5, "minimally worse," 6, "much worse," 7, "very much worse." A lower global score indicates a more positive change in clinical status.

The PDQ-39 (Hagell and Nilsson 2009) is a PD-specific health status questionnaire comprising 39 items, which assesses how often patients with PD experience difficulties across 8 dimensions of daily living including relationships, social situations, and communication, as well as the impact of PD on specific dimensions of functioning and wellbeing. Respondents are asked to mark 1 of the 5 ordered response categories (never, occasionally, sometimes, often, and always [or cannot do at all]) according to how often, due to their PD, they have experienced the problem defined by each of the 39 items.

The Starkstein Apathy Scale (Starkstein *et al* 1992) is used to identify clinically significant symptoms of apathy in patients at an early or middle stage of PD. Apathy is one of the most common neuropsychiatric features of PD, with a prevalence ranging from 17% to 70% (Pluck and Brown 2002; Aarsland *et al* 2009; Pedersen *et al* 2009). Clinically, apathy is identified as a reduction of goal-directed behavior because of a lack of feeling, interest, emotional reactivity, and motivation (Marin 1991). The Starkstein Apathy Scale is a 14-item questionnaire with a 4-point Likert Scale ranging from 3="not at all" to 0="a lot" for items 1-8; and ranging from 0="not at all" to 3="a lot" for items 9-14. The global score ranges from 0 to 42, with a higher score equal to more severe apathy.

The Clinical Global Impressions Scale (Guy 1976) initially consists of 3 different global measures: severity of illness (CGI-S); global improvement (CGI-I); and efficacy index (CGI-E). It covers 4 domains: change in clinical status (CGI-C), disease severity (CGI-S), disease improvement (CGI-I) or efficacy index (CGI-E) (1 item). CGI-S is scored on a 7-point Likert Scale from 1 (normal) to 7 (among the most severely ill patients); CGI-I is scored on a 7-point Likert Scale from 1 (very much improved) to 7 (very much worse); CGI-E requires a rating of the interaction of therapeutic effectiveness and adverse reactions; it is scored by domains and globally. Higher scores indicate greater severity of illness. The CGI-C questionnaire is used to assess clinically significant change as determined by an independent skilled and experienced clinician. The CGI-C focuses on the clinician's observations of change in the subject's cognitive, functional, and behavioral performance since the beginning of a trial. It relies on direct examination of the subject and takes into account a subject's overall function in the cognitive, behavioral, and functional activity domains. Scoring is based on examination of the subject by an independent evaluator, without consulting other information such as cognitive test results. The CGI-C requires the assessor to consider a number of cognitive, functional, and behavioral areas before providing an overall "global" assessment of clinical change. Interviews take approximately 20 minutes each.

The HADS (Zigmond and Snaith 1983) is a 14-item nonweighted, self-administered questionnaire used to detect states of anxiety and depression in adults. Anxiety and depression domains are scored on a 4-point Likert/Likert-type Scale; a lower score indicates a better quality of life.

In one embodiment, the immunogenic composition reduces pathological alpha synuclein, more particularly alpha synuclein aggregates in a subject during and/or following treatment of the immunogenic composition. This may be measured by any suitable means, for example by imaging or by analysing a suitable sample taken from the subject. A comparison may be made
5 between the subject before and during/after treatment.

In one embodiment, the immunogenic composition improves DaT-SPECT imaging results in a subject during and/or following treatment of the immunogenic composition. Again, a comparison may be made between the subject before and during/after treatment.

10

Dopamine Transporter-Single Photon Emission Computerized Tomography Imaging (DaT-SPECT) can be used to confirm or exclude a diagnosis of dopamine deficient parkinsonism in cases where the diagnosis is unclear. It can also detect the dopaminergic dysfunction in pre-symptomatic subjects at risk for PD since the reduced radiotracer binding to DaTs in striatum is
15 already present in the prodromal stage of PD (Akdemir *et al* 2021). A radioactive agent containing the active substance ioflupane (123I) is injected into the subject's bloodstream and is tracked using SPECT —a noninvasive scanner that uses radiation detectors to image the cells that take up the agent and emit radioactivity. Optionally, DaT-SPECT assessments can also be complemented with Magnetic Resonance Imaging Scans.

20

In one embodiment, the immunogenic composition improves Movement Disorder Society- Unified Parkinson's Disease Rating Scale (MDS-UPDRS) score performance in a subject during and/or following treatment with the immunogenic composition.

25 The MDS-UPDRS (Goetz *et al* 2007) is a revision of the UPDRS, which was developed to evaluate various aspects of PD including nonmotor and motor experiences of daily living and motor complications. It includes a motor evaluation and characterizes the extent and burden of disease across various populations. The scale can be used in a clinical setting as well as in research. There are 65 items covering signs and symptoms, physical functioning and
30 psychological functioning (including coping). It is scored by domains on a 5-point Likert/Likert-type Scale ranging from 0: "Normal" to 4: "Severe," and a Categorical Rating Scale ranging from 0: "Asymptomatic" to 4: "Wheelchair bound or bedridden unless aided." A higher domain score indicates more severe disease. MDS-UPDRS Part III score can be assessed in practically defined "OFF" state. Absolute values or change from baseline in MDS-UPDRS Part III score can

be used over a defined time period by treatment group within cohort and across cohorts, where applicable.

5 In one embodiment, the immunogenic composition improves advanced Magnetic Resonance Imaging (scan) results in a subject during and/or following treatment with the immunogenic composition.

10 Brain MRI scans can be examined for evidence of any brain pathology. Scans can be systematically analyzed for the presence of new brain lesion(s) or the aggravation of previous brain lesion(s) by the central reading company. Effective assessment of scans is by a qualified clinician.

15 The immunogenic compositions of the invention may be administered only once but are typically administered to a subject in a repeated dose administration regime. In one embodiment, the immunogenic composition is administered to a subject at least 3, 4, 5 or 6 times and preferably at least 6 times.

20 Where the immunogenic compositions are administered in a repeated dose regime, each administration of the immunogenic composition to a subject is at least 4 weeks apart from any preceding administration of the immunogenic composition. In some embodiments, the immunogenic composition is administered to a subject in weeks 0, 4, 12, 24, 48 and 74, preferably on days 1, 29, 85, 169, 337 and 519.

25 Typically, the immunogenic composition is administered to a subject at the same dose for each administration.

30 The immunogenic composition may be administered to a subject with an antigenic peptide dose that is between around 10 µg to around 100 µg, around 15 µg to around 75 µg or around 16 µg to around 75 µg. In a preferred embodiment, the immunogenic composition is administered to a subject with an antigenic peptide dose that is around 16 µg or around 75 µg, more preferably around 75 µg. As presented herein, the dose of the antigenic peptide is expressed with reference to a peptide incorporating an N-terminal cysteine. Therefore, the dose of the antigenic peptide is expressed with reference to CDQPVLPD (SEQ ID NO: 5).

The immunogenic compositions of the invention have been shown to generate a favourable antibody response in combination with safety, as demonstrated by administration to non-human primates (NHP, Example 3 herein) at the doses described herein. Repeated administration of the immunogenic compositions of the invention was safe and well tolerated in NHP and resulted in a substantial antibody response binding to the pathological species of alpha synuclein. Preliminary results in human clinical trials also are indicative of safety. Thus, the medical uses and treatment methods of the invention can be performed safely in a subject. Therefore, in one aspect, the invention provides an immunogenic composition of the invention for use in inducing an immune response against pathological alpha synuclein, more particularly aggregated alpha synuclein in a subject without inducing toxicity. Toxicity related observations may include mortality, clinical signs, body weight, ophthalmoscopy, ECG/BP, clinical pathology and immunophenotyping. The induced immune response is beneficial for preventing, alleviating or treating a range of conditions associated with pathological alpha synuclein, more particularly with alpha synuclein aggregates, in particular synucleinopathies, as described in detail herein.

In one aspect, the invention provides a kit for use in vaccination comprising an immunogenic composition defined herein. The immunogenic composition may be provided as a unit dose in the kit. Optionally, the kit further comprises a second immunogenic composition defined herein, which may also be provided as a unit dose. In some embodiments, the kit comprises at least six doses of immunogenic compositions according to the invention, which may be provided as unit doses. Alternatively, the kit may comprise a volume containing multiple individual doses in a single vessel. The kit may further comprise instructions for use. Such instructions may detail the route of administration, optionally together with other details of the administration regime such as the timing of multiple doses as appropriate. Preferably, the kit is for use in vaccination of a human subject.

The kit may comprise (i) a container (e.g., an injection device); (ii) a immunogenic composition within the container; and (iii) a document instructing that the immunogenic composition be administered according to a desired dosage regimen. Additionally, a label, a syringe, an injection needle, a pharmacologically acceptable medium, an alcohol cotton cloth, plaster, and the like may be additionally packaged, as appropriate, in the kit. The container may be a bottle, a glass bottle, or a syringe, for example, and may be made of any of various materials such as glass and plastics. The container contains the immunogenic composition, and has an outlet sealed with a rubber stopper, for example. The container is provided with, for example, a label indicating that the

immunogenic composition is for use in preventing or treating the relevant condition (synucleinopathy).

The immunogenic compositions of the invention when administered to a subject result in the production of antibodies with advantageous properties. Thus, the invention also provides polyclonal antibodies produced by immunising a subject with an immunogenic composition according to the invention. The antibodies may be isolated/purified from a sample obtained from the subject. A monoclonal antibody sequence may be cloned from suitable cells taken from a subject and the antibody synthesised using techniques known in the art.

Description of the Figures

Figure 1: Blockage of alpha synuclein propagation *ex vivo*. Blockage of alpha synuclein propagation shown as the percent aggregate inhibition (pS129)/[MAP2] normalized to predose) in an *ex vivo* neuron seeding assay by antibodies treated with 1/100, 1/200 or 1/500 diluted serum from ACI-7104.056 immunized NHP (normalised against pre-treated serum from NHP). Higher inhibition of aggregation is seen at lower serum dilutions. Data shown are the mean $\pm$ SEM.

Figure 2: Non Human Primate dosing and sampling schedule. Figure 2 shows a dosing schedule for ACI-7104.056 and control treated NHP. NHP were injected on days 1, 29, 57, 85, 113, 141 and 169 followed by a recovery phase. Blood samples were collected prior to day 1 (pre-bleed) and on days 8, 22, 36, 50, 64, 78, 92, 106, 120, 148, 162 and 176 (study phase) and on days 190, 204 and 211 (recovery phase).

Figure 3: Kinetics of the induced anti-PD01 (antigen) IgG antibody response after monthly immunizations with ACI-7104.056 in NHPs. Anti-PD01 IgG titers (arbitrary units "AU"/mL) as geometric mean plotted by days post first immunization. Placebo group is light grey line (circles), ACI-7104.056 low dose is dark grey line (triangles), ACI-7104.056 high dose is black line. Arrows indicate the immunization days. Data shown as the geometric mean $\pm$ 95% confidence interval (CI), with n= 10 during dosing phase for groups 2 (low) and 3 (high) and n=8 for control group (n=4 in the recovery period for all groups). Statistical analysis 1 way ANOVA in log transformed data: *=p<0.05; ***=p<0.001 in grey: Group 3 vs Group 1 AND Group 2 vs Group 1. In black: Group 3 vs Group 2 (Group 1 excluded of the analysis).

Figure 4: Kinetics of the induced anti-alpha synuclein peptide (aa115-121) IgG antibody response after monthly immunizations with ACI-7104.056 in NHPs. Anti-alpha synuclein peptide (aa115-121) IgG titers (AU/mL) as geometric mean plotted by days post first immunization. Arrows indicate injection days. Placebo group is light grey line (circles), ACI-7104.056 group 2 (low dose) is dark grey line (triangles), ACI-7104.056 group 3 (high dose) is black line. Data shown as the geometric mean $\pm$ 95% confidence interval (CI), with n= 10 during dosing phase for groups 2 (low) and 3 (high) and n=8 for control group (n=4 in the recovery period for all groups). Statistical analysis (only until Day 176). Group 2 vs Group 1 (in grey) and Group 3 vs Group 1 (in black): two-way repeated measurements ANOVA model was fitted to log-transform data using only Group 2 and Group 3. LS means and related variability were retrieved for each time point and used to assess difference to Placebo level using a one-sample two-sided t-test. Results were reported as uncorrected p-values. ***=p<0.001. Group 2 vs Group 3: repeated measurement 2-Way ANOVA in log transformed data with an uncorrected LSD Fisher's test (Group 1 excluded of the analysis).

15

Figure 5: Kinetics of the induced anti-alpha synuclein aggregate IgG antibody response after monthly immunizations with ACI 7104.056 in NHPs. Anti-alpha synuclein aggregate IgG titers (AU/mL) as geometric mean plotted by days post first immunization. Arrows indicate injection days. Placebo group is light grey line (circles), ACI-7104.056 group 2 (low dose) is dark grey line (triangles), ACI-7104.056 group 3 (high dose) is black line. Data shown as the geometric mean $\pm$ 95% confidence interval (CI), with n= 10 during dosing phase for groups 2 (low) and 3 (high) and n=8 for control group (n=4 in the recovery period for all groups). Statistical analysis (only until Day 176).

Group 2 vs Group 1 (in grey) and Group 3 vs Group 1 (in black): two-way repeated measurements ANOVA model was fitted to log-transform data using only Group 2 and Group 3. LS means and related variability were retrieved for each time point and used to assess difference to Placebo level using a one-sample two-sided t-test. Results were reported as uncorrected p-values. **=p<0.01, ***=p<0.001. Group 2 vs Group 3: repeated measurement 2-Way ANOVA in log transformed data with an uncorrected LSD Fisher's test; *=p<0.05 (in black #) (Group 1 excluded of the analysis).

30

Figure 6: ACI-7104.056 induced antibody binding profile in NHPs at different timepoints. ACI-7104.056 induced antibody preference for alpha synuclein aggregates over alpha synuclein monomers plotted as transformed ECL (Y axis) against nM concentration (X axis). Figure 6a is

from a NHP low dose (16µg) treated animal and Figure 6b is from a NHP high dose (75µg) treated animal. Serum was obtained from NHP treated animals at day 36, 120 or 176. Black line is alpha synuclein monomers. Grey line is alpha synuclein aggregates.

- 5 **Figure 7: ACI-7104.056 induced antibody binding profile in high dose treated NHPs.** ACI-7104.056 induced antibody preference for alpha synuclein aggregates over alpha synuclein monomers plotted as transformed ECL (Y axis) against nM concentration (X axis). Data expressed as mean $\pm$ SD of N = 7 NHPs from group 3 (high dose, 75 µg) on day 120. Black line is alpha synuclein monomers. Grey line is alpha synuclein aggregates.

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- Figure 8: Immunofluorescence staining of disease brain sections with sera collected from NHP before and after high dose ACI-7104.056 treatment.** Figure 8a: A pool of NHP sera from high dose group collected before (A1-A3, predose) and after immunization (n=4, day 36) (B1-B3) was used to stain alpha synuclein in frozen brain section of PDD donor. Immunofluorescence staining for the ACI-7104.056 antibody from serum (left panel), aberrant alpha synuclein (pSer129, middle panel) and a merge with DAPI staining (right panel). Figure 8b: A pool of NHP sera from high dose group collected before (A1-A3) and after immunization (n=4, day 36) (B1-B3) was used to stain alpha synuclein in frozen brain section of MSA donor. Immunofluorescence staining for the ACI-7104.056 antibody from serum (left panel), aberrant alpha synuclein (pSer129, middle panel) and a merge with DAPI staining (right panel).
- 15
- 20

- Figure 9: Phase 2 clinical trial protocol overview.** VacSyn adaptive biomarker-based Phase 2 study of ACI-7104.056 in early PD. Placebo-controlled Phase 2 Study Overview provided as Part 1: safety and Pharmacokinetics/Pharmacodynamics and Part 2: Proof of Concept in early PD.
- 25 Figure footnotes: 1. Parkinson's Disease, 2. Pharmacokinetics and Pharmacodynamics, 3. Participants must have idiopathic PD and be stable on up to 300 mg of L-Dopa treatment or treatment naïve and dopaminergic deficit determined by Dopamine Transporter Single Photon Emission Computed Tomography, 4. Alpha synuclein, 5. Proof of concept, 6. Unified Parkinson's Disease rating scale, 7. Dopamine Transporter Single Photon Emission Computed Tomography,
- 30 8. Arterial spin labelling, 9. Diffusion tensor imaging.

Figure 10: Kinetics of the induced anti-PD01 (SEQ ID NO:2) IgG antibody response after three immunizations with ACI-7104.056 in early PD subjects: Geometric Mean of Anti-PD01

IgG titers (AU/mL) +/- standard error over 14 weeks for Cohort 1, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations.

5 **Figure 11: Kinetics of the induced anti-PD01 (SEQ ID NO:2) IgG antibody response after three immunizations with ACI-7104.056 in early PD subjects:** Group Mean of Anti-PD01 IgG titers (AU/mL) +/- standard error (SEM) over 14 weeks for Cohort 1, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations.

10 **Figure 12: Kinetics of the induced anti-alpha synuclein peptide (SEQ ID NO:1) IgG antibody response after three immunizations with ACI-7104.056 in early PD subjects :** Geometric Mean of Anti-alpha-synuclein peptide IgG titers (AU/mL) +/- standard error over 14 weeks for Cohort 1, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations..

15 **Figure 13: Kinetics of the induced anti-alpha synuclein peptide (SEQ ID NO:1) IgG antibody response after three immunizations with ACI-7104.056 in early PD subjects:** Group Mean of Anti-alpha-synuclein peptide IgG titers (AU/mL) +/- standard error (SEM) over 14 weeks for Cohort 1, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations.

20

Figure 14: Kinetics of the induced anti-PD01 (SEQ ID NO:2) IgG antibody response after four immunizations with ACI-7104.056 in early PD subjects: Geometric Mean of Anti-PD01 IgG titers (AU/mL) +/- standard error over 26 weeks for Cohorts 1 & 2, per protocol group (active treatment ACI-7104.056 75µg or placebo) plotted on a logarithmic Y-axis.

25

Figure 15: Kinetics of the induced anti-PD01 (SEQ ID NO:2) IgG antibody response after four immunizations with ACI-7104.056 in early PD subjects: Group Arithmetic Mean of Anti-PD01 IgG titers (AU/mL) +/- standard error over 26 weeks for Cohorts 1 & 2, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations.

30

Figure 16: Kinetics of the induced anti-alpha synuclein peptide (SEQ ID NO:1) IgG antibody response after four immunizations with ACI-7104.056 in early PD subjects: Geometric Mean of Anti-alpha-synuclein peptide IgG titers (AU/mL) +/- standard error over 26 weeks for Cohorts 1

& 2, per protocol group (active treatment ACI-7104.056 75µg or placebo) plotted on a logarithmic Y-axis.

Figure 17: Kinetics of the induced anti-alpha synuclein peptide (SEQ ID NO: 1) IgG antibody response after four immunizations with ACI-7104.056 in early PD subjects: Group Arithmetic Mean of Anti-alpha-synuclein peptide IgG titers (AU/mL) +/- standard error over 26 weeks for Cohorts 1 & 2, per protocol group (active treatment ACI-7104.056 75µg or placebo). Arrows indicate immunizations.

The invention will be further understood with reference to the following non-limiting examples:

Examples

EXAMPLE 1: ACI-7104.056

ACI-7104.056 is a therapeutic vaccine that targets misfolded alpha synuclein and is a candidate treatment for Parkinson's Disease. ACI-7104.056 contains the PD01 antigenic peptide (DQPVLPD, SEQ ID NO: 2) which is a mimotope of the native alpha synuclein sequence at positions 115-121 (DMPVDPD, SEQ ID NO: 1). The PD01 antigenic peptide further has an N-terminal cysteine to form a stable thioether linkage with a GMBS linker connected to the CRM197 carrier protein. ACI-7104.056 is formulated for administration with an aluminium hydroxide adjuvant at 0.2%.

EXAMPLE 2: BLOCKAGE OF ALPHA SYNUCLEIN PROPAGATION IN VIVO

Primary neuronal seeding assay for assessment of efficacy in prevention of alpha synuclein aggregation and/ or propagation. Potency of elicited antibody response following immunization of NHP with ACI-7104.056 in preventing alpha synuclein aggregation was evaluated in a cellular model. In this model, rat primary neurons were exposed to human alpha synuclein preformed fibrils (hPFF), in the presence or absence of immunized sera. The addition of hPFF triggers the formation of *de novo* aggregates by recruiting endogenous rat alpha synuclein. Staining for alpha synuclein phosphorylated at serine 129 (pS129) was used as a surrogate for *de novo* aggregate detection and quantification. The reduction of alpha synuclein pS129-positive inclusions in the presence of ACI-7104.056-immunized sera was used to evaluate the capacity of elicited antibody response to block the propagation of alpha synuclein aggregates.

Method: Rat primary neurons plated in 96-well plates at seeding density of 30,000 cells/well. The hPFF were prepared starting from recombinant human alpha synuclein protein at concentration of 1mg/mL in buffer composed of Tris(hydroxymethyl)aminomethane (Tris) 50mM (pH7.4) NaCl 150mM, which was incubated for 7 days at 37°C under shaking at 1000RPM. After 7 days the sample was centrifuged at speed of 20000g at 4°C for 1 hour and the pellet was resuspended in Phosphate buffered saline (PBS), sonicated at 75 amplitude for 20 minutes at 4°C and stored as single-use aliquots in -80°C until use.

On the day of the experiment the hPFF were sonicated again, as described above, and incubated (0.5µg/mL final concentration per well) at room temperature for 20 minutes with diluted (1/100, 1/200, 1/500) NHP serum collected on D36 post-immunization (post-dose), or diluted serum of the same NHP collected before immunization (pre-dose), then the mixture was added to the neuron cultures at day *in vitro* (DIV) 6. At DIV 15, the cells were fixed and stained for Microtubule-associated protein 2 (MAP2) and alpha synuclein pS129 and imaged.

Data processing: Alpha synuclein pS129 and MAP2 channels were segmented and the total area of alpha synuclein pS129 and MAP2 per image was determined. The aggregates-to-cell ratio was defined as the aggregate area per MAP2 area (average of 9 fields of view per well):

$$ratio = \frac{area\ aggregates}{area\ neurons}$$

The aggregates-to-cell ratio used to determine the inhibition of *de novo* aggregate formation. The normalized inhibition was calculated for each condition using the following formula:

$$normalized\ inhibition = 100 - \left(\frac{ratio_{serum\ post-dose} * 100}{ratio_{serum\ pre-dose}} \right)$$

As can be seen from Figure 1 sera from ACI-7104.056-immunized NHP leads to dilution-dependent reduction of alpha synuclein aggregates within neurons demonstrating that active immunization with ACI-7104.056 elicits polyclonal response that inhibits propagation of aggregates in neuronal cells, further supporting ACI-7104.056 for therapeutic use in the

prevention and/or treatment of diseases, disorders or abnormalities associated with aggregated alpha synuclein. Figure 1 shows the mean $\pm$ SEM of three independent experiments normalized to predose of the treated NHP. Sera from ACI-7104.056-immunized NHP leads to dose dependent reduction of alpha synuclein aggregates within neurons. ACI-7104.056 elicited antibodies reduce the number of intracellular alpha synuclein aggregates in an *ex vivo* neuronal seeding assay.

EXAMPLE 3: TOXICOLOGY AND IMMUNOGENICITY IN NON HUMAN PRIMATES

Immunogenicity study of ACI-7104.056 vaccine in cynomolgus monkeys (NHP). The immunogenicity of the ACI-7104.056 vaccine was evaluated in cynomolgus monkeys at two different doses: 16 (low group 2) and 75 μ g (high group 3) (net PD01 peptide/animal/administration).

Materials and methods: A 26 week repeat dose toxicity study was performed with ACI-7104.056 vaccine. Control treatment or two doses of ACI-7104.056 (16 and 75 μ g net PD01 peptide/animal/administration) were administered intramuscularly (i.m) to male and female cynomolgus monkeys using 7 repeated 4-weekly immunization schedule (on days 1, 29, 57, 85, 113, 141, and 169). The reversibility of potential findings was evaluated during a 4-week recovery period (see Table 1 and Figure 2).

Table 1. Design of the repeat dose toxicity study with ACI-7104.056 in NHP

Group	PD01 dose level	Dose volume	Number of Animals		Necropsy after	
	(μ g/injection)	(mL/animal)	Males (M)	Females (F)	26 weeks	30 weeks
1 Control*	0	0.5	4	4	2M / 2F	2M / 2F
2 Low ACI-7104.056	16	0.5	5	5	3M / 3F	2M / 2F
3 High ACI-7104.056	75	0.5	5	5	3M / 3F	2M / 2F

* Control treatment consists of PBS buffer containing 0.2% Alhydrogel® adjuvant

Blood for antibody titer analysis was collected once during the pre-dose and 1 and 3 weeks after each immunization corresponding to days 8, 22, 36, 50, 64, 78, 92, 106, 120, 134, 148, 162, and

176 during the dosing phase, and days 190, 204, and 211 of the recovery phase as shown in Figure 2.

5 There were no adverse effects for mortality, clinical signs, body weight, ophthalmoscopy, electrocardiogram (ECG)/ blood pressure (BP), clinical pathology, immunophenotyping or organ weights for ACI-7104.056 treated NHPs.

10 There were no unscheduled deaths nor ACI-7104.056-related systemic clinical signs. Some sporadic and transient local reactions were seen in only the ACI-7104.056 treated animals and were consistent with the immunogenicity of the vaccine. There were no ACI-7104.056-related changes in body weights, ophthalmology, electrocardiology parameters, arterial blood pressure, clinical pathology parameters (including hematology, coagulation, clinical chemistry and urinary parameters), and immunophenotyping. Overall, the administration of ACI-7104.056 by intramuscular injection once every 4 weeks for 26 weeks (i.e. 7 immunizations in total) was well
15 tolerated in male and female cynomolgus monkeys at levels of 16 and 75 µg PD01/animal/administration. Microscopic findings were observed at ≥ 16 µg PD01/animal/administration at injection sites and in draining lymph nodes at the end of treatment and recovery euthanasia. They were consistent with the pharmacological activity of ACI-7104.056 and were not considered as adverse. Based on these results and under the conditions of this study, the No
20 Observed Adverse Effect Level (NOAEL) was considered to be 75 µg PD01/animal/administration, which corresponds to the highest dose tested.

Immunogenicity: Anti-PD01 IgG titers were determined in serum samples of all animals at all timepoints during the study and recovery phase.

25

Anti-human alpha synuclein peptide (aa115-122) and anti-human alpha synuclein aggregates were evaluated at pre-dose and one week after each immunization of active treatment (day 8, 36, 64, 92, 120, 148 and 176) and of recovery phase (day 204).

30 For anti-PD01 immunoglobulin G (IgG) and anti-human alpha synuclein peptide, Meso Scale Discovery (MSD) 96-well small spot streptavidin microplates were saturated overnight at 4°C. After washing, plates were coated with either biotinylated PD01 peptide or biotinylated alpha synuclein peptide (aa115-122) and incubated for one hour. After washing, standard, quality control (QC) samples and serum study samples (tested at 4 or 8 2-fold dilutions) were transferred

to the MSD plate and incubated for two hours allowing any anti-PD01 IgG antibodies or anti-alpha synuclein peptide IgG antibodies present in the samples to bind to the immobilized PD01 or alpha synuclein peptide. Plates were then washed and incubated for one hour with a Sulfo-TAG labelled anti-human IgG antibody. After washing, a substrate solution was added to the well and plates were immediately read on the MesoScale instrument. The antibody concentration in the samples was calculated from a standard curve using a four-parameter logistic fit with $1/y^2$ weighting using the MSD software and expressed in arbitrary unit/mL (AU/mL).

For anti-human alpha synuclein aggregates (*in vitro* generated oligomeric human alpha synuclein, HNE stabilized, prepared according to the protocol described by Kumar *et al.* 2020), MSD 96-well small spot standard microplates were prewetted with PBS, which was removed just before adding the coating solution. Plates were coated with human alpha synuclein aggregates overnight at 4°C. After washing plates were blocked for one hour. After washing, standard, QC samples and serum study samples (tested at 4 or 8 2-fold dilutions) were transferred to the MSD plate and incubated for two hours to allow any anti-alpha synuclein aggregates antibodies present in the samples to bind to the immobilized alpha synuclein aggregates. Plates were then washed and incubated for one hour with Sulfo-TAG labelled anti-human IgG antibody. After washing, plates were fixed with 1% formaldehyde for 15 minutes. After washing, a substrate solution was added to the well and plates were immediately read on the MesoScale instrument. The antibody concentration in the samples was calculated from the standard curve using a four-parameter logistic fitting with $1/y^2$ weighting using the MSD software and expressed in arbitrary unit/mL (AU/mL).

Competition with alpha synuclein aggregates and monomers: To evaluate the preferential binding of ACI-7104.056 induced antibodies towards alpha synuclein aggregates versus alpha synuclein monomers, competitive inhibition assay was first tested with serum of 2 animals (immunized with ACI-7104.056 at low and high dose) at different timepoints: one week after the 2nd immunization (day 36), the 5th immunization (day 120) and the 7th immunization (day 176). Additionally, competitive inhibition assay was performed in 7 animals immunized with ACI-7104.056 at 75 µg dose on day 120 (one week after the 5th immunization).

Briefly, MSD 96-well small spot standard microplates were coated with alpha synuclein aggregates overnight at 4 °C. After washing, plates were blocked for 1 hour at 37°C with agitation.

During the blocking time, in low binding 96-well plates, monoclonal antibodies (used as positive control of the assay, data not shown) or post-immunization serum samples were mixed with 3-fold serial dilutions of alpha synuclein monomers or aggregates and incubated for 45 min at room temperature under agitation. The starting concentration for both alpha synuclein species was 50
5 µg/mL. The concentration for the monoclonal antibodies was 0.1 µg/ml and the dilution for the serum samples was 1/200.

After 1 hour of blocking of the MSD plates, plates were washed, and the sera + alpha synuclein species mixture were transferred to the MSD plate and incubated for 25 min at room temperature.
10 Plates were then washed and incubated for one hour at 37°C with Sulfo-TAG labelled anti-human IgG antibody. After washing, a substrate solution was added to the well and plates were immediately read on the MesoScale. The intensity of the emitted light (expressed as ECL values) measured by the MSD reader is proportional to the quantity of anti alpha synuclein aggregates IgG present in serum samples and not bound to the monomers or alpha synuclein aggregates in
15 solution.

For the analysis, the blank was subtracted from each ECL value and the ECL value of each well was normalized against the ECL value of the well with neat sera (with no incubation with alpha synuclein monomers or aggregates in solution), which was set at 100%. The normalized values
20 were then plotted obtaining two curves per serum sample or monoclonal antibody: one with alpha synuclein monomer or the other one with alpha synuclein aggregates competition. IC50 was calculated for both curves, allowing the comparison of alpha synuclein species preferences of the vaccine-induce antibodies. Calculations were based on an assumed 30 alpha synuclein monomers per aggregate (Lorenzen et al., J Am Chem Soc. 2014 Mar 12;136(10):3859-68).

25

Co-staining immunofluorescence (IF): Frozen human brain sections from a Parkinson's disease dementia (PDD) donor and from a Multiple System Atrophy (MSA) donor were used for IF. Sections were removed from the freezer and let to dry for 30 minutes at RT. Tissue was fixed with 4% formaldehyde (FA) for 10 minutes at 4°C and washed. Then, an antigen retrieval step
30 was performed with 70% Formic acid for five minutes and washed immediately after. After blocking for one hour with 10% normal goat serum, sections were incubated overnight in a humid chamber with rabbit anti-pSer129 monoclonal antibody (targeting alpha synuclein in pSer129 position, used as positive control of pathological alpha synuclein) and the day after they were revealed with a goat anti-rabbit IgG AF-488. Brain sections were incubated with pool of serum

from monkeys immunized with ACI-7104.056 at 75 µg dose (n=4) at predose or day 36 overnight and the following day were incubated with a mouse anti-monkey IgG for 45 minutes and goat anti-mouse IgG AF-555 antibodies for 45 minutes. After a washing step, sections were incubated for 15 minutes with 0.1% Sudan black in 70% Ethanol. After a final wash, all sections were mounted using the Prolong antifade Mounting medium (with DAPI). Slides were then dried overnight and images were acquired with a Panoramic Scan P150 (3D HISTECH) and analysed with the software CaseViewer (3D HISTECH).

Results: immunogenicity. All animals immunized with both doses of ACI-7104.056 showed anti-PD01 IgG titers starting from day 22 (i.e., three weeks after the 1st immunization). Slightly higher anti-PD01 IgG titers were observed in animals receiving the 75 µg dose as compared to 16 µg dose, reaching significance on day 120 (i.e., one week after the 5th immunization) (Figure 3). In the control treated animals, no anti-PD01 IgG were observed.

To characterize the capacity of the ACI-7104.056-induced antibodies in NHP to bind human alpha synuclein, the reactivity against an alpha synuclein peptide containing the alpha synuclein human sequence and against alpha synuclein human aggregates was evaluated using the serum of animals from the study. Samples were evaluated at pre-dose and one week after each immunization of active treatment (day 8, 36, 64, 92, 120, 148 and 176) and of recovery phase (day 204).

As shown in Figures 4 and 5, monkeys immunized with both doses of ACI-7104.056, showed anti-alpha synuclein peptide IgG titers and anti-alpha synuclein aggregates IgG titers. No differences were observed between the two doses tested. In the control treated animals, no relevant IgG signal was observed against the alpha synuclein peptide or alpha synuclein aggregates. As seen from Figure 5 a strong IgG response against alpha synuclein aggregates was observed already after two immunizations and maintained at a high level until study end.

Competitive inhibition assay: To evaluate the preferential binding of ACI-7104.056 induced antibodies towards alpha synuclein aggregates versus alpha synuclein monomers, a competitive inhibition assay was first tested with serum of 2 animals (immunized with ACI-7104.056 at low and high dose) at different timepoints: one week after the 2nd immunization (day 36), the 5th immunization (day 120) and the 7th immunization (day 176). As shown in Figure 6a and 6b, ACI-7104.056 induced antibodies from NHP immunized with low dose (Figure 6a) or high dose (Figure

6b) preferentially bind to alpha synuclein aggregates over monomeric alpha synuclein. This recognition profile towards alpha synuclein aggregates was constantly maintained over time. Table 2 with calculated IC50 for each condition. Calculations were based on an assumed 30 alpha synuclein monomers per aggregate (Lorenzen et al., J Am Chem Soc. 2014 Mar 12;136(10):3859-68).

Table 2: Low and high dose induced antibodies IC50 for alpha synuclein monomers and alpha synuclein aggregates.

	ACI-7104.056 16 µg dose			ACI-7104.056 75 µg dose		
	Day 36	Day 120	Day 176	Day 36	Day 120	Day 176
IC50 nM monomers	>3457	>3457	>3457	>3457	>3457	>3457
IC50 nM aggregates	1.131	1.41	1.35	2.98	2.99	Between 1.42-4.27

Furthermore, 7 animals of high dose group (75 µg dose) were analyzed with serum from day 120. As shown in Figure 7, ACI-7104.056 induced antibodies with at least 3-log preferential recognition to alpha synuclein aggregates. Mean IC50 value for the alpha synuclein aggregates was established at 1.24 nM, ranging from 0.29 nM to 2.4 nM. Table 3 shows minimum, maximum and average for the calculated IC50. For the alpha synuclein monomers, IC50 was established as > 3457 nM.

Table 3: High dose induced antibody IC50 for alpha synuclein monomers and alpha synuclein aggregates.

	ACI-7104.056 75 µg dose		
	Min IC50	Max IC50	Average
IC50 nM monomers	>3457	>3457	>3457
IC50 nM aggregates	0.29	2.4	1.24

Pool of sera of monkeys before (predose) and after immunization with ACI-7104.056 at 75 µg dose on day 36 was used to perform IF on frozen brain slides of PDD or MSA donors (as described

above). Sections were co incubated with pSer129 monoclonal antibody used as positive control for pathological alpha synuclein. As shown in Figure 8a (PDD donor) and 8b (MSA donor), pool sera at predose showed no signal (Figure 8a - A1 and 8b - A1), while the pool serum from monkeys immunized with ACI-7104.056 vaccine showed a binding to alpha synuclein present in the brain slides (Figure 8a - B1 and Figure 8b - B1) that co-stain with the positive control pSer129 antibody (single staining Figure 8a-B2 and Figure 8b-B2, and co-staining Figure 8a - B3 and Figure 8b - B3). These results further demonstrate that elicited antibodies bind to pathological alpha synuclein present in human PDD and MSA brain tissue.

10 **EXAMPLE 4: Phase 2 trial with ACI-7104.056 vaccine in humans**

ACI-7104.056 has been selected for an adaptive, Phase 2, double-blind, randomized, placebo-controlled, multicenter study to evaluate the safety, tolerability, immunogenicity, and pharmacodynamic effects of ACI-7104.056 in patients with early stages of Parkinson's disease. ACI-7104.056 is being tested in a Phase 2 clinical trial, NCT06015841. The study vaccine ACI-7104.056 is an optimized version of the vaccine PD01A.

OBJECTIVES AND END POINTS

Primary Objectives:

- 20 To assess the safety and tolerability of the study vaccine (ACI-7104.056).
- To assess the antibody response induced by the study vaccine in serum.

Secondary Objectives:

- 25 To assess the pharmacodynamic effect of the study vaccine on alpha synuclein (alpha synuclein) related blood and/or cerebrospinal fluid (CSF) biomarkers including pathogenic alpha synuclein oligomer species.
- To assess the effect of the study vaccine on Dopamine Transporter-Single Photon Emission Computerized Tomography (DaT-SPECT) imaging.
- To assess the clinical effects of the study vaccine on Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) Part III score.
- 30

Exploratory Objectives:

To explore the effect of the study vaccine on brain using advanced magnetic resonance imaging (MRI).

To explore the effect of the study vaccine on neuromotor functions (MDS-UPDRS), and on nonmotor functions (including cognitive functions with MDS-UPDRS and additional clinical scales).

To explore the subject's need for dopaminergic symptomatic treatment during the study by determination of cumulative levodopa, L-3,4-Dihydroxyphenylalanine (L-Dopa) equivalent dose.

To explore the effect of the study vaccine on patient-reported outcome and quality of life assessment scales. To explore other biomarkers in any collected blood and/or CSF sample (eg, omics-related expression, neurodegeneration, neuroinflammation, and immunogenicity) associated with Parkinson's disease (PD) and related to the study vaccine.

Primary Endpoints:

Safety and Tolerability: Adverse events; physical and neurological examination results; global assessment of tolerability; vital signs; brain MRI; electrocardiogram; routine laboratory tests (eg, hematology and biochemistry evaluation) in blood and urine; suicidality as measured with the Columbia Suicide Severity Rating Scale.

Immunogenicity: Antibody response induced by the study vaccine.

Secondary Endpoints:

Biological Effect/Pharmacodynamics/Clinical effect:

Absolute levels and change from baseline in alpha synuclein related fluid biomarkers, including pathogenic alpha synuclein oligomer species and/or differentially phosphorylated synuclein species, in any collected blood and/or CSF sample.

Change from baseline in DaT-SPECT at 48 weeks and 100 weeks.

Absolute values and change from baseline in the MDS-UPDRS Part III score over 100 weeks.

Exploratory Endpoints:

Total MDS-UPDRS sum scores and individual combinations of MDS-UPDRS items as determined by item analysis or compound scores utilizing different scales and measures.

Absolute measures and change from baseline in raw and/or processed data on nonmotor Parkinson features as determined by the MDS Nonmotor Rating Scale or by additional clinical scales.

Treatment effect on structural and advanced MRI measures.

5 Absolute values and change from baseline in assessment scales for clinical, cognitive, and quality of life.

Absolute levels and change from baseline in exploratory biomarkers related to PD, neurodegeneration, neuroinflammation, and immunogenicity which are related to ACI-7104.056 in any collected blood and/or CSF sample.

10 L-Dopa cumulative additional dose during the study.

Inclusion criteria:

1. Confirmed diagnosis of clinically established early idiopathic PD using the modified Movement Disorder Society criteria, after excluding any other known or suspected cause of PD. The presence of motor symptoms should not be of more than 2 years at screening.
2. Monotherapy treatment with L-Dopa at 300 mg per day, with a stable dose prior to baseline for 3 months. The subject has a reasonably low likelihood of requiring dose adjustment within the next 6 to 12 months after enrollment. Any exception to this rule has to be previously agreed with the Sponsor medical monitor.
3. Male or female.
4. Aged ≥ 40 to ≤ 75 years.
5. Body weight range of ≥ 45 kg to ≤ 110 kg (99 to 242 lbs) and a body mass index of ≥ 18 to ≤ 34 kg/m².
6. Modified Hoehn-Yahr Stage I to II.
7. A centrally read screening brain DaT-SPECT consistent with PD.
8. Subjects can understand the informed consent form, are able and willing to provide written informed consent, and can be expected to comply with the study protocol according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use and local regulations.
9. Female subjects must be post-menopausal for at least 1 year and/or surgically sterilized, or, if they are women of childbearing potential or not post-menopausal, they must have a negative blood pregnancy test at screening and be willing to use highly effective methods of contraception from the screening visit until the end of the safety follow-up period (approximately 108 weeks). Male subjects in the trial with female partners

of childbearing potential are required to use barrier methods of contraception (condoms with spermicide) in addition to contraceptive measures used by female partners during the whole study duration. Men must refrain from donating sperm during this same period. The female partners of male subjects should use a highly effective method of contraception with a failure rate of less than 1% per year from screening until the end of the safety follow-up period (approximately 108 weeks). The reliability of sexual abstinence should be evaluated in relation to the duration of the clinical trial and the preferred and usual lifestyle of the subject. Periodic abstinence (eg, calendar, ovulation, symptothermal, or postovulation methods) and withdrawal are not acceptable methods of contraception.

Exclusion criteria:

1. Medical history indicating a Parkinsonian syndrome other than idiopathic PD, including but not limited to, progressive supranuclear palsy, multiple system atrophy, drug-induced parkinsonism, essential tremor, vascular parkinsonism, primary dystonia.
2. Known carriers of certain familial PD gene mutations (PRKN, PINK1, DJ1, LRRK2).
3. History of PD-related freezing episodes or falls.
4. History of brain surgery or any neurosurgical procedures.
5. Reside in a nursing home or assisted care facility.
6. A history of cancer within 5 years of baseline with the exception of fully excised nonmelanoma skin cancers or nonmetastatic prostate cancer that has been stable for at least 6 months, or cervical intraepithelial neoplasia stage I uterine cancer.
7. History of and/or screening brain MRI scan indicative of, clinically significant abnormality including but not limited to prior hemorrhage or infarct >1 cm³ or >3 lacunar infarcts.
8. Diagnosis of a significant central nervous system disease other than PD (including but not limited to, Huntington's disease, normal pressure hydrocephalus, cerebrovascular disease including stroke, fronto-temporal dementia, Alzheimer's disease, dementia with Lewy bodies, multiple sclerosis, brain tumor); history of repeated head injury; history of epilepsy or seizure disorder other than febrile seizures as a child.
9. Presence of psychiatric symptoms (eg, confusion, hallucination, delusion, excitation, delirium, abnormal behavior at screening and baseline). Note: mild depression, depressive mood, or mild anxiety arising in the context of PD are not exclusionary.
10. Clinically significant concomitant disease or condition within 6 months prior to screening, or as specified below, that could interfere with the conduct of the study, or that would, in the opinion of the investigator, pose an unacceptable risk to the study subject:

- a. Autoimmune disease (except well controlled conditions as specified in the study protocol with decision making on a case per case basis).
- b. Any active infectious disease and/or any febrile illness (including non-infectious) within 1 week prior to first dose administration.
- 5 c. Any current psychiatric diagnosis that may interfere with the subject's ability to perform the study.
- d. Female subjects who are pregnant or breastfeeding or intending to become pregnant during the study.
- e. Myocardial infarction within 12 months of baseline.
- 10 f. Known history or documentation of uncontrolled hypotension or bradycardia on more than 1 occasion within 3 months prior to baseline and known history or documentation of uncontrolled hypertension on more than 1 occasion within 3 months prior to baseline (up to Principal Investigator's discretion). Resting pulse rate >100 or <45 bpm. A QT interval corrected using Fridericia's formula
- 15 measurement of 450 ms for males or 470 ms for females at screening or a family history of long QT-syndrome. Intermittent second or third degree atrioventricular (AV) heart block or AV dissociation or any other clinically significant cardiovascular disease.
- g. Clinically significant abnormalities in laboratory test results at the screening visit (specified in more detail in a separate document), positive result for acute or
- 20 chronic infectious Hepatitis B virus (HBV; [ie, Hepatitis B surface antigen (HBsAg) positive test]), for Hepatitis C virus (HCV), or for Human Immunodeficiency Virus (HIV) 1 or 2. Successfully treated subjects with HCV (undetectable HCV RNA) are eligible for enrollment. Subjects who are immune due to HBV natural infection or
- 25 HBV vaccination are eligible.
- h. Use of any of the following: monoamine oxidase (MAO)-B inhibitors, catechol-O-methyl transferase (COMT) inhibitors (eg, entacapone, opicapone, tolcapone), amantadine, adenosine A2a antagonists or anticholinergics, or dopaminergic medication (both ergot and nonergot [pramipexole, ropinirole,
- 30 rotigotine] dopamine agonists) for more than a total of 90 days or within 90 days of baseline.
- i. Antiepileptic medication (eg, valproate) for nonseizure-related treatment that has not remained stable for at least 60 days prior to baseline.

- 5 j. Antidepressant use that has not remained stable for at least 2 months prior to baseline. Use of any of the following medications within 90 days prior to baseline: metoclopramide, alpha methyl dopa, flunarizine, amoxapine, amphetamine derivatives, reserpine, bupropion, buspirone, cocaine, cannabis, mazindol, methamphetamine, methylphenidate, phentermine, phenylpropanolamine, and modafinil. Exceptional use of norephedrine is allowed (eg, treatment for a cold).
- 10 l. Previous participation in a clinical trial using an active immunotherapy against PD, unless there is firm evidence that the subject received placebo only and the placebo formulation is not expected to induce any specific immune response.
- m. Previous treatment with any investigational and/or marketed passive immunotherapy against PD within 6 months before screening or 5 half-lives, whichever is longer, unless there is firm evidence that the subject received placebo only (in the case of any IMP administered within the frame of a clinical trial participation).
- 15 n. Participation in previous clinical trials for PD and/or for neurological disorders using any small molecule drug with a washout <30 days or <5 half-lives of the drug, whichever is longer, before screening, unless there is firm evidence that the subject received placebo only.
- 20 o. Concomitant participation in any other clinical trial using experimental or approved medications or therapies (eg, device, stem cell). This does not include noninterventional devices for disease tracking or imaging studies.
- p. Immunomodulating and immunosuppressant drugs including oral corticosteroids within 30 days prior to baseline.
- 25 q. Allergy to any of the components of the study vaccine.
- r. Any contraindications to obtaining a brain MRI or DaT-SPECT, or getting a lumbar puncture or receiving IM injections.
11. Current, or history of, alcohol or drug (including cannabis) abuse or other dependence (except nicotine dependence) within 12 months before screening.
12. Subjects with known hypersensitivity to the study vaccine or placebo components.
- 30 13. Subjects who previously received a vaccination (ie, influenza vaccine and COVID-19) within the last 4 weeks prior to randomization, or standard-of-care immunizations (eg, tetanus, herpes zoster, pneumococcal pneumonia) within the last 2 weeks prior to randomization.

14. Subjects being treated with any anticoagulants or antiplatelet drugs, except aspirin at doses of 100 mg daily or lower.

OVERALL DESIGN

5 This is a Phase 2, multicenter, placebo-controlled, double-blind, randomized study with adaptive features, comprising a screening period of up to 8 weeks, a 74-week double-blind treatment period, and a 26-week post-treatment follow-up period to evaluate the safety, tolerability, and immunogenicity of the study vaccine, ACI-7104.056.

10 After the screening period, 16 PD subjects are randomized in the first cohort (Cohort 1) to receive either the study vaccine or placebo administered intramuscularly (IM) in a 3:1 active treatment/placebo ratio.

Additional cohorts of 16 PD subjects using a different administration regimen and/or dose may be

15 initiated to optimize the antibody response and/or safety/tolerability.

The overall design is summarised in Figure 9. Study overview.

Based on supportive interim analysis (IA) data on safety/tolerability, one of the initial potential
20 three cohorts (Cohorts 2 and 3 are optional) may be expanded to up to 32 subjects as described in "Number of Subjects" below.

For cohort expansion, the initial 3:1 ratio is maintained for the first 32 subjects. For cohort expansion, the active treatment/placebo ratio is adjusted to reach a 2:1 ratio in this cohort, to further evaluate the pharmacodynamic and clinical effects (efficacy, safety/tolerability) of the study
25 vaccine.

Multiple data interim analyses (supported by DSMB recommendation) are being performed during the study.

30 NUMBER OF PARTICIPANTS

Up to 3 cohorts to include 16 subjects each (12 under the study vaccine and 4 under placebo; 3:1 active treatment/placebo ratio). One of the initial potential 3 cohorts (Cohorts 2 and 3 are optional) can be expanded in order to reach an overall total of up to 150 subjects in the study.

In case a cohort is expanded, the initial 3:1 ratio will be maintained for the first 32 subjects. If the cohort is further expanded, the active treatment/placebo ratio will be adjusted to reach a 2:1 ratio in this cohort.

- 5 Subjects with early stages of idiopathic PD with an age range of 40 to 75 years old.

INTERVENTION GROUPS

The route of administration of the study vaccine and placebo are intramuscular injections.

- 10 Study cohort(s)

The dose of the study vaccine to be tested in the first cohort (Cohort 1) is 75 µg.

Randomized PD subjects are receiving 6 injections of the study vaccine or placebo, at Week 0 (baseline), and Weeks 4, 12, 24, 48, and 74.

- 15 For each study subject, the treatment period (74 weeks) is being followed by a 26-week post-treatment follow-up period. The overall subject participation is approximately 108 weeks (up to 8 weeks of screening, 74 weeks of treatment, and 26 weeks of follow-up).

SAFETY EVALUATIONS

- 20 Key safety assessments include solicited local and systemic AEs, unsolicited AEs, SAEs, physical examinations, vital sign measurements, and clinical laboratory tests.

IMMUNOGENICITY EVALUATIONS

Antibody response induced by the study vaccine. Multiple IAs of immunogenicity data is being conducted in each cohort, either expanded or not, in some or in all subjects, during the study treatment period and the post-treatment follow-up period.

STATUS

- 30 ACI-7104.056, is being tested in a Phase 2, multicenter, placebo-controlled, double-blind, randomized study (VacSyn study; NCT06015841). The first dosing was performed in July 2023 with the randomization of 34 early PD subjects in April 2024. As of 09 August 2024, 1 subject has received 5 injections (weeks 0, 4, 12, 24 and 48), 23 subjects have received 4 injections (weeks 0, 4, 12 and 24), 9 subjects have received 3 injections (weeks 0, 4 and 12) and 1 subject has received 2 injections (weeks 0 and 4).

As of 08 November 2024, 8 subjects have received 5 injections (weeks 0, 4, 12, 24 and 48), 23 subjects have received 4 injections (weeks 0, 4, 12 and 24), 2 subjects have received 3 injections (weeks 0, 4 and 12) and 1 subject has received 2 injections (weeks 0 and 4).

Patient demographics for the 34 randomized patients are provided below (Table 4).

5

Table 4: Patient Demographics

Baseline profile	Unit	Total ¹
Total number of patients	n	34
Age	Years mean (std)	62.1 (6.7)
Sex		
Male	n (%)	22 (65%)
Female	n (%)	12 (35%)
Hoehn and Yahr stage		
Stage I	n (%)	16 (47%)
Stage II	n (%)	18 (53%)
MDS-UPDRS scores		
Part 1: Non-motor experiences of daily living	mean (std)	4.09 (3.1)
Part 2: Non-motor experiences of daily living	mean (std)	4.09 (3.2)
Part 3: Motor examination	mean (std)	21.09 (9.8)
PD Treatment		
treatment-naïve	n (%)	11 (32%)
on L-Dopa 300mg/day	n (%)	23 (68%)

To-date, the ACI-7104.056 vaccine has shown good safety and tolerability, with no clinically relevant safety issues reported. No deaths, no serious adverse events or no severe adverse events have been reported. One adverse event (AE) leading to discontinuation from the study has been reported, this being worsening of a pre-existing generalized anxiety disorder, considered unrelated to the study drug (active or placebo).

15

The most commonly reported AEs were local injection site reactions generally of mild severity, observed in 50% of the pooled active or placebo subjects, and headaches (in all cases mild) in 14.7% of the pooled active or placebo subjects. No safety concerns have been raised to date.

5

In an interim analysis to week 14 in the first 16 patients (cohort 1), an increase in both anti-PD01 IgG titers (Figures 10 and 11) and anti-alpha-synuclein peptide IgG titers (Figures 12 and 13) was apparent following two immunizations from week six onwards. A more pronounced increase of titers occurred in these patients following the third immunization, indicating a boosting effect. No increase in anti-PD01 IgG or anti-alpha-synuclein peptide IgG titers was observed in placebo-treated subjects. As seen from Figure 13, ACI-7104.056-treated patients showed an average 16-fold increase above placebo in anti-alpha-synuclein antibodies after 3 immunizations. At 14 weeks a 100% responder rate was observed for anti-PD01 IgG, and a 66.7% responder rate was observed for anti-alpha-synuclein peptide IgG.

10
15

In a subsequent interim analysis to week 26 in 34 patients (cohorts 1 & 2), an increase in both anti-PD01 IgG titers (Figures 14 and 15) and anti-alpha-synuclein peptide IgG titers (Figures 16 and 17) was also apparent following two immunizations from week six onwards. A more pronounced increase of titers occurred in these patients following the third and fourth immunizations, indicating a boosting effect and the potential to further increase antibody titers. As seen from Figure 17, treatment with ACI-7104.056 induced an increase in anti-a-syn antibodies on average over 20-fold higher than placebo after 4 immunizations.

20

For anti PD01 IgG titers a responder is defined here by increase in AU of at least 3.21-fold from baseline. For anti a-syn peptide IgG titers a responder is here defined by an increase in AU of at least 3.02-fold from baseline.

25

To date, preliminary clinical results showed that ACI-7104.056 induced a high, specific and boostable antibody response toward alpha-synuclein. Active immunization with ACI-7104.056 is well tolerated and no clinically relevant safety concerns have been reported to date. These results support the further development of ACI-7104.056 as a disease-modifying treatment for synucleinopathies such as Parkinson's disease, MSA and DLB.

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REFERENCES

- Aarsland et al., J Neurol Neurosurg Psychiatry. 2009 Aug;80(8):928-30.
- Akdemir et al., Turk J Med Sci. 2021 Apr 30;51(2):400-10.
- Berg et al., Mov Disord. 2018 Oct;33(10):1643-6.
- 5 Braak et al., Neurobiol. Aging. 2003; 24:197–211.
- Chaudhuri et al. Mov Disord. 2020 Jan;35(1):116-33.
- Cookson, Annu. Rev. Biochem., 2005, 74, 29-52.
- Crowther et al., FEBS Lett 1998, 436(3), 309-312.
- Dorval et al., J Biol Chem. 2006, 281(15):9919-24.
- 10 Fanciulli et al., N. Engl. J. Med. 2015; 372, 249–63.
- Fujiwara et al., Nat Cell Biol 2002, 4(2), 160-164.
- Galvin et al., Arch Neurol. 2001, 58,186-90.
- Goetz et al., Mov Disord. 2007 Jan;22(1):41-7.
- Guy W (ed) (1976). ECDEU Assessment Manual for Psychopharmacology. Rockville, MD: US
- 15 Department of Health, Education, and Welfare Public Health Service Alcohol, Drug Abuse, and Mental Health Administration.
- Hashimoto et al, Neuron. 2001 Oct 25;32(2):213-23.
- Hagell et al., Ther Adv Neurol Disord. 2009 Jul;2(4):205-14.
- Hasegawa et al., J Biol Chem 2002, 277(50), 49071-49076.
- 20 Hermanson. Bioconjugate Techniques 3rd edition (Hardback ISBN: 9780123822390
eBook ISBN: 9780123822406).
- Ischiropoulos et al., Ann N Y Acad Sci. 2003, 991, 93-100.
- Iwai et al., Biochemistry 1995, 34(32), 10139-10145.
- Kaur et al. Alzheimer Dis Assoc Disord. 2018 Apr-Jun;32(2):120-4.
- 25 Kumar et al., Neurobiology of Disease. 146 (2020) 105086: 1-22.
- Lashuel et al., J. Mol. Biol., 2002, 322, 1089-102.
- Lesage et al., Hum. Mol. Genet., 2009, 18, R48-59.
- Li et al., Proc Natl Acad Sci USA 2005, 102(6), 2162-2167.
- Lorenzen et al., J Am Chem Soc. 2014 Mar 12;136(10):3859-68
- 30 Luk et al., Science. 2012, 338(6109):949-5.
- Tran et al., Cell Rep. 2014, 7(6):2054-65).
- Mandler et al., Acta Neuropathol. 127 (2014), 861.
- Mandler et al., Molecular Neurodegeneration 10 (2015),10: 1-15.

- Marotta et al., *Chembiochem*. 2012;13:2665–2670.
- McLean et al., *Neurosci Lett* 2002, 323(3), 219-223.
- Munch et al., *J Chem Neuroanat*. 2000;20:253–257.
- Nasreddine et al. *J Am Geriatr Soc*. 2005 Apr;53(4):695-9.
- 5 Negro et al., *FASEB J* 2002, 16(2), 210-212.
- Oueslati et al., *Prog Brain Res* 2010, 183, 115-145.
- Outeiro et al., *Mol. Neurodegener*. 2019, 14, 5.
- Pagonabarraga et al. *Mov Disord*. 2008 May 15;23(7):998-1005.
- Pluck et al. *J Neurol Neurosurg Psychiatry*. 2002 Dec;73(6):636-42.
- 10 Rochet et al., *Biochemistry* 2000, 39(35), 10619-10626.
- Ruzafa et al., *PlosOne* 2017 12(5):e0178576.7Santangelo et al. *Neurol Sci*. 2017 May;38(5):845-53.
- Schmid et al., *J Biol Chem* 2009, 284(19), 13128-13142.
- Pedersen et al. *Parkinsonism Relat Disord*. 2009 May;15(4):295-9.
- 15 Starkstein et al. *J Neuropsychiatry Clin Neurosci*. 1992 Spring;4(2):134-9.
- Stefanova et al., *Neuropathol. Appl. Neurobiol*. 2016, 42, 20-32.
- Takahashi et al., *Brain Res* 2002, 938(1-2), 73-80).
- Vigneswara et al., *PLoS One* 8 (2013), e61442.
- Volc et al., *Lancet Neurol* 2020, 19: 591-600.
- 20 Volles et al., *Biochemistry* 2002, 41(14), 4595-4602.
- Wood et al., *J Biol Chem* 1999, 274(28), 19509-19512.
- Zigmond et al. *Acta Psychiatr Scand*. 1983 Jun;67(6):361-70.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings

25 as commonly understood by one of ordinary skill in the art to which this invention belongs. All publications and patents specifically mentioned herein are incorporated by reference in their entirety for all purposes in connection with the invention.

The present invention is not to be limited in scope by the specific embodiments described herein.

30 Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art from the foregoing description and accompanying figures. Such modifications are intended to fall within the scope of the appended claims. Moreover, all aspects and embodiments of the invention described herein are considered to be broadly applicable and

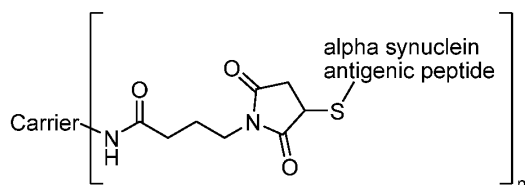
combinable with any and all other consistent embodiments, including those taken from other aspects of the invention (including in isolation) as appropriate.

CLAIMS

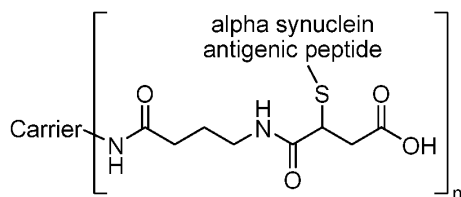
1. An immunogenic composition comprising a conjugate of:
 - a. an alpha synuclein antigenic peptide that is a mimotope of the native alpha synuclein amino acid sequence DMPVDPD (SEQ ID NO: 1), wherein the antigenic peptide comprises or consists of the amino acid sequence DQPVLPD (SEQ ID NO: 2); and
 - b. a carrier protein comprising a detoxified form of diphtheria toxin, wherein the number of alpha synuclein antigenic peptides conjugated to each carrier protein is between 10 and 24.
2. The immunogenic composition of claim 1, wherein the number of alpha synuclein antigenic peptides conjugated to each carrier protein is a mean value of between 12 and 14.
3. The immunogenic composition of claim 2 or claim 3, wherein when administered to a subject the immunogenic composition induces the production of antibodies that preferentially bind to alpha synuclein aggregates over alpha synuclein monomers.
4. The immunogenic composition of claim 3, wherein the antibodies have an IC50 for alpha synuclein aggregates of around 5 nM or less, around 4.5 nM or less, around 4.27 nM or less, around 4 nM or less, around 3.5 nM or less, around 3 nM or less, around 2.99 nM or less, around 2.98 nM or less, around 2.5 nM or less, around 2.4 nM or less, around 2 nM or less, around 1.5 nM or less, around 1.41 nM or less, around 1.35 nM or less, around 1.24 nM or less, around 1.131 nM or less, around 1 nM or less, around 0.5 nM or less or around 0.29 nM or less, preferably as measured by competitive Meso Scale Discovery (MSD) assay.
5. The immunogenic composition of claim 3 or claim 4, wherein the antibodies have an IC50 for alpha synuclein monomers of around 2500 nM or more, around 3000 nM or more, or around 3457 nM or more, preferably as measured by competitive Meso Scale Discovery (MSD) assay.
6. The immunogenic composition of any one of claims 3 to 5, wherein the antibodies have:
 - a. an IC50 for alpha synuclein aggregates of around 5 nM or less, around 4.5 nM or less, around 4.27 nM or less, around 4 nM or less, around 3.5 nM or less, around 3 nM or less, around 2.99 nM or less, around 2.98 nM or less, around 2.5 nM or less, around 2.4 nM or less, around 2 nM or less, around 1.5 nM or less, around 1.41 nM or less, around 1.35 nM or less, around 1.24 nM or less, around

- 1.131 nM or less, around 1 nM or less, around 0.5 nM or less or around 0.29 nM or less, preferably as measured by competitive Meso Scale Discovery (MSD) assay; and
- b. an IC₅₀ for alpha synuclein monomers of around 2500 nM or more, around 3000 nM or more, or around 3457 nM or more, preferably as measured by competitive Meso Scale Discovery (MSD) assay.
7. The immunogenic composition of any one of claims 3 to 6, wherein the antibodies have an IC₅₀ for alpha synuclein monomers that is at least 500-fold, at least 1000-fold, at least 2500-fold, at least 5000-fold, at least 7500-fold, at least 10000-fold or at least 11920-fold, greater than the IC₅₀ for alpha synuclein aggregates.
8. The immunogenic composition of any one of claims 3 to 7, wherein the antibodies delay or inhibit the propagation of pathological alpha synuclein, particularly alpha synuclein aggregates.
9. The immunogenic composition of any one of claims 3 to 8, wherein the antibodies either:
- inhibit and/or delay seeding of alpha synuclein; and/or
 - inhibit and/or delay spontaneous alpha synuclein aggregation; and/or
 - inhibit and/or delay spreading of aggregated alpha synuclein; and/or
 - prevent cell to cell spreading of pathological alpha synuclein, in particular aggregated alpha synuclein.
10. The immunogenic composition of any one of claims 3 to 9, wherein the antibodies reduce the number of intracellular alpha synuclein aggregates at least 10%, 15% or 25%, preferably as measured in serum by a neuron seeding assay.
11. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide is 7 to 30, 7 to 20, 7 to 16, 8 or 7 amino acid residues in length.
12. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide consists of the amino acid sequence DQPVLPD (SEQ ID NO: 2).
13. The immunogenic composition of any one of claims 1 to 11, wherein the antigenic peptide consists of the amino acid sequence DQPVLPDN (SEQ ID NO: 3) or DQPVLPDG (SEQ ID NO: 4).
14. The immunogenic composition of any one of the preceding claims, wherein the detoxified form of diphtheria toxin is a genetically detoxified form.
15. The immunogenic composition of any one of the preceding claims, wherein the detoxified form of diphtheria toxin is CRM197.

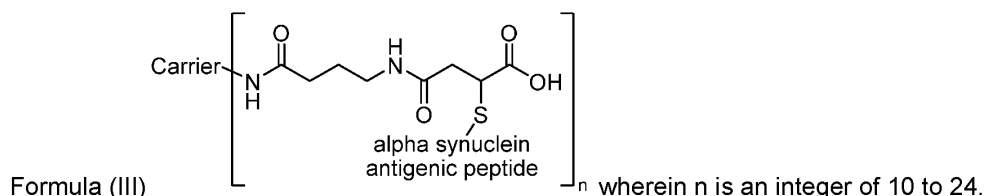
16. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide is conjugated with the carrier protein via a linker.
17. The immunogenic composition of claim 16, wherein a cysteine residue is incorporated in the antigenic peptide to facilitate reaction with the linker.
18. The immunogenic composition of claim 17, wherein the cysteine residue is attached to the N-terminus of the antigenic peptide, preferably wherein the antigenic peptide consists of the amino acid sequence CDQPVLPD (SEQ ID NO: 5).
19. The immunogenic composition of any one of claims 16 to 18, wherein the linker comprises a chemical linker.
20. The immunogenic composition of any one of claims 16 to 19, wherein the linker is a heterobifunctional linker, preferably comprising a NHS ester and a maleimide reactive group.
21. The immunogenic composition of any one of claims 16 to 20, wherein the linker comprises N-γ-maleimidobutyryl-oxy succinimide ester (GMBS).
22. The immunogenic composition of any one of the preceding claims, wherein the conjugate has the structure of:



Formula (I) wherein n is an integer of 10 to 24 ; and/or



Formula (II) wherein n is an integer of 10 to 24 ; and/or



23. The immunogenic composition of any one of the preceding claims, wherein the concentration ratio on a grams per unit volume basis of antigenic peptide to carrier protein is around 1:2 to 1:50, preferably around 1:3 to 1:6.
24. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide is in a concentration of around 20-200 µg/ml relative to a carrier protein concentration of around 400-1000 µg/ml.
25. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide is present in a concentration of around 150 µg/ml relative to a carrier protein concentration of around 582-825 µg/ml.
26. The immunogenic composition of any one of the preceding claims, comprising between around 16 and around 75 µg antigenic peptide, preferably 75 µg antigenic peptide.
27. The immunogenic composition of any one of the preceding claims, comprising between around 290 and around 420 µg of carrier protein.
28. The immunogenic composition of any one of the preceding claims in a volume of 0.5 ml.
29. The immunogenic composition of any one of the preceding claims further comprising an adjuvant.
30. The immunogenic composition of claim 29, wherein the adjuvant is aluminium hydroxide.
31. The immunogenic composition of claim 30, wherein the aluminium hydroxide is hydrated, preferably in the form of a hydrogel.
32. The immunogenic composition of any one of claims 29 to 31, comprising 0.5 mg adjuvant.
33. The immunogenic composition of any one of the preceding claims, wherein the immunogenic composition further comprises one or more buffers.
34. The immunogenic composition of claim 33, wherein the one or more buffers comprise NaCl, KH₂PO₄, Na₂HPO₄ and/or KCl, preferably NaCl, KH₂PO₄, Na₂HPO₄ and KCl.
35. The immunogenic composition of claim 34, wherein the Na₂HPO₄ is a dodecahydrate.
36. The immunogenic composition of claim 33 or claim 34, wherein the NaCl is present in a concentration of around 5-10 mg/ml, the KH₂PO₄ is present in a concentration of around

0.1-0.5 mg/ml, the Na_2HPO_4 is present in a concentration of around 2-4 mg/ml, and the KCl is present in a concentration of around 0.1-0.5 mg/ml.

37. The immunogenic composition of any one of the preceding claims, wherein the antigenic peptide is contained in an amount from 0.1 ng to 10 mg, preferably 10 ng to 1 mg, in particular 100 ng to 100 μg .
38. The immunogenic composition of any one of the preceding claims in the form of a vaccine.
39. An immunogenic composition of any one of the preceding claims for use in the prevention, alleviation or treatment of a condition associated with alpha synuclein, particularly pathological alpha synuclein, more particularly with alpha synuclein aggregates.
40. The immunogenic composition for use of claim 39, wherein the condition is a synucleinopathy.
41. The immunogenic composition for use of claim 40, wherein the synucleinopathy is selected from the group consisting of Parkinson's disease (PD) (sporadic, familial with alpha synuclein mutations and/or copy number variations, familial with mutations other than alpha synuclein, pure autonomic failure and Lewy body dysphagia), Lewy Body dementia (LBD; dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), Parkinson's disease dementia (PDD)), Diffuse Lewy Body Disease (DLBD), sporadic Alzheimer's disease, familial Alzheimer's disease with APP mutations, familial Alzheimer's disease with PS-1, PS-2 or other mutations, familial British dementia, Lewy body variant of Alzheimer's disease, multiple system atrophy (MSA) (Shy-Drager syndrome, striatonigral degeneration and olivopontocerebellar atrophy), inclusion-body myositis, traumatic brain injury, chronic traumatic encephalopathy, dementia pugilistica, tauopathies (Pick's disease, frontotemporal dementia, progressive supranuclear palsy, corticobasal degeneration, Frontotemporal dementia with Parkinsonism linked to chromosome 17 and Niemann-Pick type C1 disease), Down syndrome, Creutzfeldt-Jakob disease, Huntington's disease, motor neuron disease, amyotrophic lateral sclerosis (sporadic, familial and ALS-dementia complex of Guam), neuroaxonal dystrophy, neurodegeneration with brain iron accumulation type 1 (Hallervorden-Spatz syndrome), prion diseases, Gerstmann-Straussler-Scheinker disease, ataxia telangiectatica, Meige's syndrome, subacute sclerosing panencephalitis, Gaucher disease, Krabbe disease as well as other lysosomal storage disorders (including Kufor-

Rakeb syndrome and Sanfilippo syndrome), or rapid eye movement (REM) sleep behavior disorder.

42. The immunogenic composition for use of claim 40 or claim 41, wherein the synucleinopathy is multiple system atrophy (MSA), dementia with Lewy bodies (DLB) ("pure" Lewy body dementia), or Parkinson's disease (PD), preferably early stage idiopathic Parkinson's Disease.
43. The immunogenic composition for use of any one of claims 39 to 42, wherein an anti-alpha-synuclein immunogenic response is induced following two administrations of the immunogenic composition.
44. The immunogenic composition for use of any one of claims 39 to 42, wherein an anti-alpha-synuclein immunogenic response is boosted as measured at least 2 weeks after the administration of a second dose of the immunogenic composition.
45. The immunogenic composition for use of claim 44, wherein the immunogenic response is increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150% or more as measured at least 2 weeks after the second administration of the immunogenic composition.
46. The immunogenic composition for use of any one of claims 39 to 42, wherein an anti-alpha-synuclein immunogenic response is boosted as measured at least 2 weeks after the administration of a third dose of the immunogenic composition.
47. The immunogenic composition for use of claim 46, wherein the immunogenic response is increased at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150% or more as measured at least 2 weeks after the third administration of the immunogenic composition.
48. The immunogenic composition for use of any one of claims 39 to 47, wherein the immunogenic composition prevents and/or slows down and/or halts and/or retains and/or improves the motor capabilities or motor deficits, cognitive capabilities or cognitive deficits, or behavioral impairments in a subject during and/or following treatment with the immunogenic composition.
49. The immunogenic composition for use of any one of claims 39 to 48, wherein the immunogenic composition improves clinical, cognitive or quality of life in a subject during and/or following treatment with the immunogenic composition as measured by one or more of MoCA, PD-CRS; MDS-NMS, PGI-C/-S, PDQ-39, Starkstein Apathy Scale, CGI-C/-S, HADS.

50. The immunogenic composition for use of any one of claims 39 to 49, wherein the immunogenic composition reduces alpha synuclein aggregates in a subject during and/or following treatment of the immunogenic composition.
51. The immunogenic composition for use of any one of claims 39 to 50, wherein the immunogenic composition improves DaT-SPECT imaging results in a subject during and/or following treatment of the immunogenic composition.
52. The immunogenic composition for use of any one of claims 39 to 51, wherein the immunogenic composition improves MDS-UPDRS score performance in a subject during and/or following treatment with the immunogenic composition.
53. The immunogenic composition for use of any one of claims 39 to 52, wherein the immunogenic composition improves advanced Magnetic Resonance Imaging results in a subject during and/or following treatment with the immunogenic composition.
54. The immunogenic composition for use of any one of claims 39 to 53, wherein the immunogenic composition is administered to a subject in a repeated dose administration regime.
55. The immunogenic composition for use of any one of claims 39 to 54, wherein the immunogenic composition is administered to a subject at least 6 times.
56. The immunogenic composition for use of any one of claims 39 to 55, wherein each administration of the immunogenic composition to a subject is at least 4 weeks apart from any preceding administration of the immunogenic composition.
57. The immunogenic composition for use of any one of claims 39 to 56, wherein the immunogenic composition is administered to a subject in weeks 0, 4, 12, 24, 48 and 74, preferably on days 1, 29, 85, 169, 337 and 519.
58. The immunogenic composition for use of any one of claims 39 to 57, wherein the immunogenic composition is administered to a subject at the same dose for each administration.
59. The immunogenic composition for use of any one of claims 39 to 58, wherein the immunogenic composition is administered to a subject with an antigenic peptide dose that is between around 10 µg to around 100 µg, around 15 µg to around 75 µg or around 16 µg to around 75 µg.
60. The immunogenic composition for use of any one of claims 39 to 59, wherein the immunogenic composition is administered to a subject with an antigenic peptide dose that is around 16 µg or around 75 µg, preferably around 75 µg.

61. The immunogenic composition for use of any one of claims 39 to 60, wherein the immunogenic composition is administered to a subject intramuscularly.
62. The immunogenic composition for use of any one of claims 39 to 60, wherein the immunogenic composition is administered to a subject subcutaneously.
63. A kit for use in vaccination comprising an immunogenic composition according to any one of claims 1 to 38.
64. A ready to use vial containing 0.5-1 ml, preferably 0.75 ml, of an immunogenic composition according to any one of claims 1 to 38.
65. The ready to use vial of claim 64 containing 0.75 ml of immunogenic composition, wherein the immunogenic composition comprises 112.5 µg antigenic peptide and 436.5-618.75 µg carrier protein, optionally wherein the immunogenic composition further comprises 0.75 mg adjuvant.
66. A monoclonal antibody or polyclonal antibodies produced by immunising a subject with the immunogenic composition according to any one of claims 1 to 38.