

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

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

Summary of the Invention:

[00018] The disclosure relates to novel monoclonal antibodies, and antigen-binding fragments thereof, capable of specifically binding an epitope within amino acids 112-

117 (**SEQ ID NO:9** (ILEDMP)) in human alpha-synuclein (**SEQ ID NO:10**). The epitope bound by the antibodies or antibody-binding fragments thereof of the disclosure, such as exemplary antibody "GM37", or "GM285", is referred to herein as "the 112-117 epitope". The antibodies of the present disclosure specifically bind to an epitope within the 112-117 epitope and may, according to one embodiment, compete with antibody GM37 or GM285 for binding to an epitope within amino acids 112-117. For example, antibodies or antigen-binding fragments thereof according to the disclosure may compete for binding to an epitope within amino acids 112-117 of human alpha-synuclein with a heavy chain consisting of a variable domain of **SEQ ID NO:7** and a light chain consisting of a variable domain of **SEQ ID NO:8**. Such competitive binding inhibition can be determined using assays and methods well known in the art, for example using an unlabelled binding assay such as surface plasmon resonance (SPR). For example, immobilising human alpha-synuclein on a surface and incubating with or without the reference antibody 'GM37' prior to incubation with an antibody or binding fragment to be tested. Alternatively, a pair-wise mapping approach can be used, in which the reference antibody 'GM37' is immobilised to the surface, human alpha-synuclein antigen is bound to the immobilised antibody, and then a second antibody is tested for simultaneous binding ability to human alpha-synuclein (see 'BIAcore® Assay Handbook', GE Healthcare Life Sciences, 29-0194-00 AA 05/2012; the disclosures of which are incorporated herein by reference).

[00019] More specifically the GM285 antibody binds an epitope within residues 112-117 of alpha-synuclein comprising residues 112-115 of alpha-synuclein (ILED; **SEQ ID NO:19**).

[00020] In one embodiment, the disclosure relates to monoclonal antibody GM37, its variants (e.g., GM37 Variant 1, GM37 Variant 2 and GM37 Variant 3), or GM285.

[00021] In particular, the disclosure provides a monoclonal antibody GM37, its variants (e.g., GM37 Variant 1, GM37 Variant 2 and GM37 Variant 3), or GM285, and encompasses such antibodies as well as derivatives thereof that possess a sufficient number (e.g., 1, 2, or 3) light chain CDRs and a sufficient number (e.g., 1, 2, or 3) heavy chain CDRs to form a binding site capable of specifically binding to human synuclein. Preferably, such antibodies will possess the three light chain CDRs and three heavy chain CDRs, as defined below. The numbering of amino acid residues in

[00026] The antibodies and antigen-binding fragments thereof of the disclosure may be used in a method to treat, diagnose or image synucleinopathies, such as Parkinson's disease ((PD), including idiopathic and inherited forms of Parkinson's disease), Diffuse Lewy Body Disease (DLBD), Lewy body variant of Alzheimer's disease (LBV), Gauchers Disease (GD), Combined Alzheimer's and Parkinson disease (CAPD), pure autonomic failure and multiple system atrophy.

[0026a] In one aspect of the disclosure, there is provided a pharmaceutical composition comprising the monoclonal antibody as disclosed herein and a pharmaceutical acceptable carrier.

Brief Description of the Drawings

[00027] Fig. 1 shows immunization protocols for generation of hybridomas. The table outlines the differences of the immunogens and mouse strains used for the identification of GM37 and GM285. Different HCo17-Balb/c and HCo12/Balb/c mice were immunized independently (description of these mice are provided below). The hybridoma expressing GM37 was identified from mice immunized with full length alpha-synuclein containing amino acids 1-140 fibrils and boosted with truncated alpha-synuclein fragments 1-60 and 1-119 of full length (FL) alpha-synuclein (**SEQ ID NO 10**). The hybridoma expressing antibody GM285 came from an immunization protocol in which HCo12-Balb/c mice were immunized with full length monomeric alpha-synuclein, amino acids 1-140 followed by a boost with full length fibrillary alpha-synuclein (**Example 1**).

[00028] Fig. 2 (**PANEL A-C**) shows screening of GM37 for binding to alpha synuclein, alpha-synuclein homologs and orthologs.

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

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

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

24. The monoclonal antibody according to claim 23, comprising a heavy chain consisting of a variable domain of **SEQ ID NO:26** and a variable domain of **SEQ ID NO:27**.
25. A pharmaceutical composition comprising the monoclonal antibody according to any one of the previous claims, and a pharmaceutical acceptable carrier.