

en.wikipedia.org/w/index.php?title=Synucleinopathies&oldid=686287116 (accessed May 30, 2017).

3. "Beta-synuclein," *Wikipedia, The Free Encyclopedia*, website address: en.wikipedia.org/w/index.php?title=Beta-synuclein&oldid=763171134 (accessed May 30, 2017).

4. "Synucleinopathies," *Wikipedia, The Free Encyclopedia*, website address: en.wikipedia.org/w/index.php?title=Synucleinopathies&oldid=686287116 (accessed May 30, 2017).

5. LEE, J.S., et al., "Mechanism of Anti- α -Synuclein Immunotherapy", *J Mov Disord.*; 9(1):14-19 (2016)

6. TRAGGIAI, E., et al. "An efficient method to make human monoclonal antibodies from memory B cells: potent neutralization of SARS coronavirus", *Nat Med.*; 10(8):871-875 (2004)

7. WANG, C., et al. "Versatile Structures of α -Synuclein", *Front Mol Neurosci.* 9:48 (2016)

SUMMARY

In one aspect, the present disclosure is directed to an alpha-synuclein (α -Syn) peptide immunogen construct comprising: a B cell epitope consisting of 10 to 25 amino acid residues from a C-terminal fragment of α -Syn corresponding to amino acid G111 to amino acid D135 of SEQ ID NO: 1; a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 83, and 84; and an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, and ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 148), wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

In another aspect, the present disclosure is directed to a composition comprising the α -Syn peptide immunogen construct of the disclosure.

In another aspect, the present disclosure is directed to a composition comprising more than one α -Syn peptide immunogen construct of the disclosure.

In another aspect, the present disclosure is directed to a pharmaceutical composition comprising the α -Syn peptide immunogen construct of the disclosure and a pharmaceutically acceptable delivery vehicle and/or adjuvant.

In another aspect, the present disclosure is directed to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the α -Syn peptide immunogen construct of the disclosure.

5 In another aspect, the present disclosure is directed to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the α -Syn peptide immunogen construct of the disclosure.

In another aspect, the present disclosure is directed to a composition comprising the isolated antibody or epitope-binding fragment thereof according to the disclosure.

10 In another aspect, the present disclosure is directed to a composition comprising the isolated antibody or epitope-binding fragment thereof according to the disclosure.

In another aspect, the present disclosure is directed to a use of the α -Syn peptide immunogen of the disclosure and a delivery vehicle and/or adjuvant in the manufacture of a medicament for producing antibodies that recognize α -Syn in a host.

15 In another aspect, the present disclosure is directed to a use of the α -Syn peptide immunogen of the disclosure in the manufacture of a medicament for inhibiting α -Syn aggregation in an animal.

In another aspect, the present disclosure is directed to a use of the α -Syn peptide immunogen of the disclosure the manufacture of a medicament for reducing the amount of α -Syn aggregates in an animal.

20 In another aspect, the present disclosure is directed to a method of identifying α -Syn aggregates of different sizes in a biological sample comprising: a. exposing the biological sample to the antibody or epitope-binding fragment thereof according to the disclosure under conditions that allow the antibody or epitope-binding fragment thereof to bind to the α -Syn aggregates; and b. detecting the amount of the antibody or epitope-binding fragment thereof bound to the α -Syn aggregates in the biological sample.

The present disclosure is directed to peptide immunogen constructs of the alpha-synuclein protein (α -Syn). The present disclosure is also directed to compositions containing the peptide immunogen constructs, methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

30 The disclosed peptide immunogen constructs contain a B cell epitope from α -Syn linked to a heterologous T helper cell (Th) epitope directly or through an optional heterologous spacer.

The B cell epitope portion of the peptide immunogen constructs contains about 10 to about 25 amino acid residues from the C-terminal region of α -Syn, corresponding to the sequence from about the Glycine at amino acid position 111 (G111) to about the Asparagine at amino acid position 135 (D135) of full-length α -Syn (SEQ ID NO: 1). The heterologous Th epitope portion
5 of the peptide immunogen constructs are derived from amino acid sequences derived from pathogenic proteins. The B cell epitope and Th epitope portions of the peptide immunogen constructs act together when administered to a host to stimulate the generation of antibodies that specifically recognize and bind to the α -Syn B cell epitope portion of the constructs.

In some examples, the α -Syn peptide immunogen construct comprises: (a) a B cell

epitope comprising about 10 to about 25 amino acid residues from a C-terminal fragment of α -Syn corresponding to about amino acid G111 to about amino acid D135 of SEQ ID NO: 1; (b) a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 70-98; and (c) an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, and ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 148), wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer. In specific examples, the α -Syn peptide immunogen construct comprises an amino acid sequence selected from the group consisting of SEQ ID Nos: 107, 108, 111 – 113, and 115 – 147.

The present disclosure is also directed to compositions containing the disclosed peptide immunogen constructs, including pharmaceutical compositions. The disclosed pharmaceutical compositions are capable of eliciting an immune response and the production of antibodies against the disclosed peptide immunogen constructs in a host. The disclosed compositions can contain one or a mixture of more than one of the disclosed peptide immunogen constructs. In some examples, the compositions contain the disclosed peptide immunogen constructs together with additional components, including carriers, adjuvants, buffers, and other suitable reagents. In certain examples, the compositions contain the disclosed peptide immunogen constructs in the form of a stabilized immunostimulatory complex with a CpG oligomer that is optionally supplemented with an adjuvant.

In some examples, the compositions comprise an α -Syn peptide immunogen construct comprises an amino acid sequence selected from the group consisting of SEQ ID Nos: 107, 108, 111 – 113, 115 – 147. In certain examples, the composition is a pharmaceutical composition comprising an α -Syn peptide immunogen construct selected from the group consisting of SEQ ID Nos: 107, 108, 111 – 113, 115 – 147 and a pharmaceutically acceptable carrier or adjuvant.

The present disclosure is also directed to antibodies that are produced by a host that is immunized with the disclosed peptide immunogen constructs. The disclosed antibodies specifically recognize and bind to the α -Syn B cell epitope portion of the peptide immunogen constructs. The disclosed α -Syn antibodies have an unexpectedly high cross-reactivity to the β -sheet of α -Syn in the form of monomers, oligomers, or fibrils. Based on their unique characteristics and properties, the disclosed antibodies are capable of providing an immunotherapeutic approach to targeting, identifying, and treating synucleinopathies.

In specific examples, the antibody or epitope-binding fragment thereof specifically binds to the B cell epitope of the α -Syn peptide immunogen construct selected from the group consisting of SEQ ID Nos: 107, 108, 111 – 113, 115 – 147.

The present disclosure is also directed to methods of making and using the disclosed peptide immunogen constructs, antibodies, and compositions. The disclosed methods provide for the low cost manufacture and quality control of peptide immunogen constructs and compositions containing the constructs, which can be used in methods for preventing and treating synopathies.

The present disclosure also includes methods for treating and/or preventing synucleinopathies using the disclosed peptide immunogen constructs and/or antibodies directed against the peptide immunogen constructs. In some examples, the methods for treating and/or preventing synucleinopathies including administering to a host a composition containing a disclosed peptide immunogen construct. In certain examples, the compositions utilized in the methods contain a disclosed peptide immunogen construct in the form of a stable immunostimulatory complex with negatively charged oligonucleotides, such as CpG oligomers, through electrostatic association, which complexes are further supplemented, optionally, with mineral salts or oil as adjuvant, for administration to patients with synucleinopathies. The disclosed methods also include dosing regimens, dosage forms, and routes for administering the peptide immunogen constructs to a host at risk for, or with, synucleinopathies.

In various examples, methods of using the α -Syn peptide immunogen construct and/or antibodies elicited by the α -Syn peptide immunogen construct are described. In specific examples, the methods are for producing antibodies, inhibiting α -Syn aggregation, reducing the amount of α -Syn aggregates, and identifying α -Syn aggregates of different sizes are described. The various methods comprise administering a pharmacologically effective amount of the α -Syn peptide immunogen to a host in need thereof.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a graph showing the level of *in vitro* α -Syn aggregation after 6 days in the presence of antibodies directed against the C-terminal end of α -Syn (Samples 1-4) or in the presence of a vehicle control (Sample 5). Specifically, α -Syn aggregation was carried out in the presence of anti- α -Syn antibodies elicited by: α -Syn₁₁₁₋₁₃₂ (Sample 1); α -Syn₁₂₁₋₁₃₅ (Sample 2); α -Syn₁₂₃₋₁₃₅ (Sample 3); α -Syn₁₂₆₋₁₃₅ (Sample 4); or a vehicle control (Sample 5). The level of α -Syn

CLAIMS

1. An alpha-synuclein (α -Syn) peptide immunogen construct comprising:
a B cell epitope consisting of 10 to 25 amino acid residues from a C-terminal fragment of α -Syn corresponding to amino acid G111 to amino acid D135 of SEQ ID NO: 1;
a T helper epitope comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 81, 83, and 84; and
an optional heterologous spacer selected from the group consisting of an amino acid, Lys-, Gly-, Lys-Lys-Lys-, (α , ϵ -N)Lys, and ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 148),
wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.
2. The α -Syn peptide immunogen construct of **claim 1**, wherein the B cell epitope is selected from the group consisting of SEQ ID NOs: 12 – 15, 17, and 49 – 63.
3. The α -Syn peptide immunogen construct of **claim 1**, wherein the optional heterologous spacer is (α , ϵ -N)Lys or ϵ -N-Lys-Lys-Lys-Lys (SEQ ID NO: 148).
4. The α -Syn peptide immunogen construct of **claim 1**, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope.
5. The α -Syn peptide immunogen construct of **claim 1**, wherein the T helper epitope is covalently linked to the amino terminus of the B cell epitope through the optional heterologous spacer.
6. The α -Syn peptide immunogen construct of **claim 1** comprising the following formula:

$$(\text{Th})_m-(\text{A})_n-(\alpha\text{-Syn C-terminal fragment})-\text{X}$$

or

$$(\alpha\text{-Syn C-terminal fragment})-(\text{A})_n-(\text{Th})_m-\text{X}$$

wherein

Th is the T helper epitope;

A is the heterologous spacer;

(α -Syn C-terminal fragment) is the B cell epitope;

X is an α -COOH or α -CONH₂ of an amino acid;

m is from 1 to about 4; and

n is from 1 to about 10.

7. The α -Syn peptide immunogen construct of **claim 1**, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 107, 108, 111 – 113, and 115 – 121.

8. The α -Syn peptide immunogen construct of **claim 1**, comprising the amino acid sequence selected from the group consisting of SEQ ID NOs: 107, 108, and 111 – 113.

9. A composition comprising the α -Syn peptide immunogen construct of **claim 1**.

10. A composition comprising more than one α -Syn peptide immunogen construct of **claim 1**.

11. The composition of **claim 10**, wherein the α -Syn peptide immunogen constructs have amino acid sequences of SEQ ID NOs: 112 and 113.

12. A pharmaceutical composition comprising the α -Syn peptide immunogen construct of **claim 1** and a pharmaceutically acceptable delivery vehicle and/or adjuvant.

13. The pharmaceutical composition of **claim 12**, wherein

a. the α -Syn peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 107, 108, 111 – 113, and 115 – 121; and

b. the adjuvant is a mineral salt of aluminum selected from the group consisting of Al(OH)₃ or AlPO₄.

14. The pharmaceutical composition of **claim 12**, wherein

a. the α -Syn peptide immunogen construct is selected from the group consisting of SEQ ID NOs: 107, 108, 111 – 113, and 115 – 121; and

- b. the α -Syn peptide immunogen construct is mixed with an CpG oligodeoxynucleotide (ODN) to form a stabilized immunostimulatory complex.
15. An isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the α -Syn peptide immunogen construct of **claim 1**.
16. The isolated antibody or epitope-binding fragment thereof according to **claim 15** bound to the α -Syn peptide immunogen construct.
17. An isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the α -Syn peptide immunogen construct of **claim 8**.
18. A composition comprising the isolated antibody or epitope-binding fragment thereof according to **claim 15**.
19. A composition comprising the isolated antibody or epitope-binding fragment thereof according to **claim 17**.
20. The composition of **claim 19**, comprising a mixture of
- a. an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of SEQ ID NO: 112; and
- b. an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of SEQ ID NO: 113.
21. Use of the α -Syn peptide immunogen of **claim 1** and a delivery vehicle and/or adjuvant in the manufacture of a medicament for producing antibodies that recognize α -Syn in a host.
22. Use of the α -Syn peptide immunogen of **claim 1** in the manufacture of a medicament for inhibiting α -Syn aggregation in an animal.
23. Use of the α -Syn peptide immunogen of **claim 1** in the manufacture of a medicament for reducing the amount of α -Syn aggregates in an animal.
24. A method of identifying α -Syn aggregates of different sizes in a biological sample comprising:

- a. exposing the biological sample to the antibody or epitope-binding fragment thereof according to **claim 15** under conditions that allow the antibody or epitope-binding fragment thereof to bind to the α -Syn aggregates; and
- b. detecting the amount of the antibody or epitope-binding fragment thereof bound to the α -Syn aggregates in the biological sample.