

and formulations thereof for cost effective treatment of patients suffering tauopathies.

References:

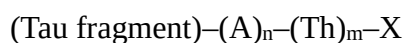
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SUMMARY

In one aspect, the present disclosure refers to a Tau peptide immunogen construct can be
10 represented by the formulae:



or



wherein

15 Th is a heterologous T helper epitope;

A is a heterologous spacer;

(Tau fragment) is a B cell epitope having about 15 to about 40 amino acid residues from the full-length Tau protein of SEQ ID NO: 100;

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

20 m is from 1 to about 4; and

n is from 0 to about 10.

In another aspect, the present disclosure refers to a Tau peptide immunogen construct comprising: a B cell epitope comprising about 15 to about 40 amino acid residues from the full-length Tau protein sequence of SEQ ID NO: 100; a T helper epitope comprising an amino acid
25 sequence selected from the group consisting of SEQ ID NOs: 22-50; 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: 102), wherein the B cell epitope is covalently linked to the T helper epitope directly or through the optional heterologous spacer.

In yet another aspect, the present disclosure refers to a composition comprising a peptide
30 immunogen construction as disclosed herein.

In yet another aspect, the present disclosure refers to a pharmaceutical composition comprising: a. a peptide immunogen construct as disclosed herein; and b. and a pharmaceutically acceptable delivery vehicle and/or adjuvant.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-
35 binding fragment thereof that specifically binds to the B cell epitope of the Tau peptide

immunogen construct as disclosed herein.

In yet another aspect, the present disclosure refers to an isolated antibody or epitope-binding fragment thereof that specifically binds to the B cell epitope of the Tau peptide immunogen construct as disclosed herein.

5 In yet another aspect, the present disclosure refers to a composition comprising the isolated antibody or epitope-binding fragment thereof as disclosed herein.

The present disclosure is directed to individual peptide immunogen constructs targeting portions of the Tau protein for the treatment and/or prevention of tauopathies. The present disclosure is also directed to compositions containing the peptide immunogen constructs,
10 methods of making and using the peptide immunogen constructs, and antibodies produced by the peptide immunogen constructs.

The disclosed peptide immunogen constructs contain about 15 or more amino acids. The peptide immunogen constructs contain a B cell epitope from portions of the longest isoform of the human Tau protein (GenBank: AGF19246.1) having the amino acid sequence of SEQ ID
15 NO: 100 shown in **Table 8**. The B cell epitope can be linked to a heterologous T helper cell (Th) epitope derived from pathogen proteins through an optional heterologous spacer. The disclosed peptide immunogen constructs stimulate the generation of highly specific antibodies directed against Tau. The disclosed peptide immunogen constructs can be used as an immunotherapy for patients suffering from tauopathies.

20 The B cell epitope portion of the peptide immunogen constructs have amino acid sequences from the full-length Tau protein (SEQ ID NO: 100). In some examples, the B cell epitope has a sequence containing any of SEQ ID NOs: 1-21 and 101-124 as shown in **Table 1**.

The peptide immunogen constructs of the present disclosure can contain a heterologous Th epitope amino acid sequence derived from a pathogenic protein (e.g., SEQ ID NOs: 22 to 50)
25 as shown in **Table 2**. In certain examples, the heterologous Th epitope is derived from natural pathogens, such as Diphtheria Toxin (SEQ ID NO: 26), Plasmodium Falciparum (SEQ ID NO: 27), Cholera Toxin (SEQ ID NO: 29). In other examples, the heterologous Th epitope is an idealized artificial Th epitope derived from Measles Virus Fusion protein (MVF 1 to 5) or Hepatitis B Surface Antigen (HBsAg 1 to 3) in the form of either single sequence or
30 combinatorial sequences (e.g., SEQ ID NOs: 33, 32, and 34).

In some examples, the peptide immunogen constructs contain a B cell epitope from Tau linked to a heterologous T helper cell (Th) epitope through an optional heterologous spacer. In certain examples, the peptide immunogen constructs contain a B cell antigenic site having

the amino acid sequence from Tau (SEQ ID NOs: 1-21 and 101-124) linked to a heterologous Th epitope derived from a pathogenic protein (e.g., SEQ ID NOs: 22 to 50) through an optional heterologous spacer. In some examples, the optional heterologous spacer is a molecule or chemical structure capable of linking two amino acids and/or peptides together. In certain examples, the spacer is a naturally occurring amino acid, a non-naturally occurring amino acid, or a combination thereof. In specific examples, the peptide immunogen constructs have the amino acid sequence of SEQ ID NOs: 51-71 and 125-155 shown in **Table 3**.

The present disclosure is also directed to compositions containing a Tau peptide immunogen construct. In some examples, the disclosed compositions contain more than one Tau peptide immunogen constructs to cover multiple B epitopes from Tau. In certain examples, the compositions contain a mixture of Tau peptide immunogen constructs (e.g., any combination of SEQ ID NOs: 51-71 and 125-155) with more than one heterologous Th epitope derived from pathogenic proteins to cover a broad genetic background in patients. Compositions containing a mixture of peptide immunogen constructs can lead to a higher percentage in responder rate upon immunization for the treatment of tauopathies compared to compositions containing only a single Th peptide immunogen construct.

The present disclosure is also directed to pharmaceutical compositions for the treatment and/or prevention of tauopathies. In some examples, the pharmaceutical compositions contain the disclosed peptide immunogen constructs in the form of a stabilized immunostimulatory complex formed through electrostatic associations by mixing a CpG oligomer with a composition containing a peptide immunogen complex. Such stabilized immunostimulatory complexes are able to further enhance the immunogenicity of the peptide immunogen constructs. In some examples, the pharmaceutical compositions contain adjuvants such as mineral salts, including alum gel (ALHYDROGEL), aluminum phosphate (ADJUPHOS), or water-in-oil emulsions including MONTANIDE ISA 51 or 720.

The present disclosure is also directed to antibodies directed against the disclosed Tau peptide immunogen constructs. In particular, the peptide immunogen constructs of the present disclosure are able to stimulate the generation of highly specific antibodies that are cross-reactive with the Tau amino acid sequences (SEQ ID NOs: 1-21 and 101-124) when administered to a subject. The highly specific antibodies produced by the peptide immunogen constructs are cross reactive with recombinant Tau-containing proteins. The disclosed antibodies bind with high specificity to Tau without much, if any, directed to the heterologous Th epitopes employed for immunogenicity enhancement, which is in sharp contrast to the conventional protein or other biological carriers used for such peptide antigenicity enhancement.

The present disclosure also includes methods for treating and/or preventing tauopathies

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 tauopathies 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 tauopathies. 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, tauopathies.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A – ELISA results expressed in Log₁₀ Titers for immune sera obtained from multiple Tau immunogen constructs at 6 weeks post initial injection (wpi) against non-phosphorylated Tau peptides when analyzed on antigenic regions of Tau.

Figure 1B – ELISA results expressed in Log₁₀ Titers for immune sera obtained from multiple Tau immunogen constructs at 6 weeks post initial injection (wpi) against phosphorylated Tau peptides when analyzed on antigenic regions of Tau.

Figure 2 – Western blot analysis of cell lysates containing monomers, dimers, trimers, and oligomers of the Tau protein. The various lanes indicate IgG antibodies elicited by the non-phosphorylated Tau peptide immunogen constructs shown above each lane (left side). These peptide immunogen constructs correspond to the constructs shown in **Table 3**. The binding profiles for each antibody was analyzed and summarized in the bar graph (right side). Representative immune sera to Tau immunogen constructs demonstrated binding reactivities to multispecies of Tau (monomers, dimer, trimers, oligomers and polymers).

Figure 3A – Graphs showing the level of full-length Tau (Tau441 amino acids) aggregation after exposure to various antibodies directed against phosphorylated Tau peptide immunogens at concentrations ranging from 0 to 5 µg/mL shown above each graph in comparison to antibodies from the preimmune sera. The level of Tau aggregation was measured by Thioflavin-T (ThT) staining of the aggregates.

Figure 3B – Bar graphs showing the level of in vitro Tau aggregation in the presence 5 µg/mL