



(51) International Patent Classification:

A61K 38/12 (2006.01) *A61P 25/28* (2006.01)
A61K 39/00 (2006.01) *C07K 19/00* (2006.01)
A61K 39/39 (2006.01) *C07K 7/64* (2006.01)
A61K 47/48 (2006.01) *C07K 14/47* (2006.01)

(21) International Application Number:

PCT/CA2015/050659

(22) International Filing Date:

14 July 2015 (14.07.2015)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

62/024,387 14 July 2014 (14.07.2014) US
 62/150,239 20 April 2015 (20.04.2015) US

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(81) Designated States (*unless otherwise indicated, for every
 kind of national protection available*): AE, AG, AL, AM,
 AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY,
 BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM,
 DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,
 HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR,
 KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG,
 MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM,
 PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC,
 SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN,
 TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (*unless otherwise indicated, for every
 kind of regional protection available*): ARIPO (BW, GH,
 GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ,
 TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU,
 TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE,
 DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU,
 LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,
 SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ,
 GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— with international search report (Art. 21(3))

(54) Title: CYCLIC PEPTIDE VACCINATION FOR TREATMENT OF AMYLOID BETA-RELATED DISORDERS

Head-to-tail cyclization of CGSNKGG

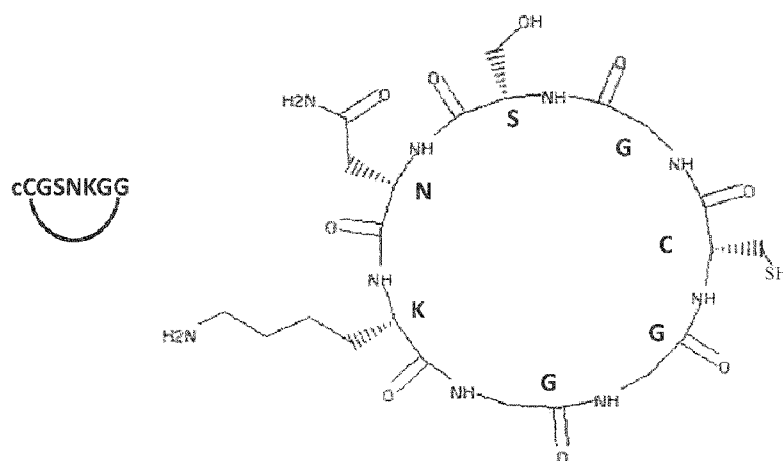


Figure 1

(57) Abstract: Pharmaceutical compositions and methods for the treatment or prophylaxis of Amyloid beta-related disorders such as Alzheimer's disease in a subject are provided by vaccination using a cyclic peptide or antibody to the SNK epitope in the knuckle region of oligomeric A β amyloid.

CYCLIC PEPTIDE VACCINATION FOR TREATMENT OF AMYLOID BETA-RELATED DISORDERS

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CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to both U.S. Provisional Application Number 62/024,387, filed July 14, 2014, and U.S. Provisional Application Number 62/150,239, filed April 20, 2015, which are incorporated herein by reference in their entireties.

BACKGROUND

[0002] The present disclosure relates to compositions and methods for the treatment and prophylaxis of amyloid beta-related diseases, disorders, or conditions, including, e.g., Alzheimer's disease. Also disclosed are compositions and methods for screening and diagnosing amyloid beta-related diseases, disorders, or conditions, including, e.g., Alzheimer's disease.

[0003] Alzheimer's disease (AD) is a common dementing (disordered memory and cognition) neurodegenerative disease. It is associated with accumulation in the brain of extracellular plaques composed predominantly of the amyloid beta peptides (also referred to as amyloid β , Abeta or A β), including, primarily, A β (1–40), A β (1–42) and A β (1–43) peptides. These A β peptides are proteolytic products of amyloid precursor protein (APP). In addition, neurofibrillary tangles, composed principally of abnormally phosphorylated tau protein (a neuronal microtubule-associated protein), accumulate intracellularly in dying neurons. The A β (1–42) is the dominant species in the amyloid plaques of Alzheimer's disease patients. A β oligomerization has been shown to be a key part of neurotoxicity in Alzheimer's disease.

[0004] It has been found that a particular molecular species of A β , in which the peptide is oligomerized, mediates the major component of neurotoxicity observed in Alzheimer's disease and mouse models of the disease (Walsh *et al.*, Naturally secreted oligomers of amyloid beta protein potently inhibit hippocampal long-term potentiation *in vivo*, Nature, 2002, 416(6880): 535-9). A β oligomer toxicity can be manifested by dysfunction of neuronal insulin receptors (Zhao *et al.*, Amyloid beta oligomers induce impairment of neuronal insulin receptors, FASEB J. 2008, 22(1):246-60), and by interference with normal synaptic function, particularly in the hippocampus, by ectopic activation of glutamatergic receptors (De Felice *et al.*, 2007. Abeta oligomers induce neuronal oxidative stress through an N-methyl-D-aspartate receptor-dependent

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mechanism that is blocked by the Alzheimer drug memantine, *J. Biol. Chem.* 282:11590–11601; Nimmrich *et al.*, Amyloid beta oligomers (Abeta(1-42) globulomer suppress spontaneous synaptic activity by inhibition of P/Q-type calcium currents, *J Neurosci.*, 2008, 23;28(4):788-97). Ahmed *et al.*, 2010, *Nature Structural & Molecular Biology*, 17:561-568. Hefti *et al.*, 2013, *Trends in Pharmacological Sciences* 34:261-266.

[0005] Amyloid plaques are generally formed via cleavage of the amyloid precursor protein (APP) by beta-secretase and gamma-secretase, to form the peptide A β (1- 42) (Hamley IW, 2012 *Chem Rev* 112:5147-5192). While the presence of insoluble amyloid plaques is one of the hallmarks of Alzheimer's disease, cognitive loss has been found to correlate poorly with the number of amyloid plaques. Instead, cognitive loss appears to better correlate with other forms of A β , for example soluble A β oligomers or aggregates, suggesting that A β oligomers could be more directly linked to neuronal and synaptic loss (Jack *et al.*, 2013, *Lancet* 12:207-216; Vos *et al.*, 2013, *Lancet Neurology* 12:957-965).

[0006] Many *in vitro* and *in vivo* studies have been conducted and the results demonstrate that immune therapy against A β can lead to the improvement of both the pathology and cognitive behavior of transgenic mice expressing human mutant APP. Unfortunately, these positive immunotherapy results in mice have not translated directly in humans as there were adverse events associated with the treatment, including autoimmune meningoencephalitis, during clinical trials (Gilman *et al.*, Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial, *Neurology*, 2005, 10;64(9):1553-62). However, there are still numerous immunotherapy treatments, both passive and active, in clinical trials.

[0007] In healthy individuals, antibodies specific to at least A β (1-42) are naturally present. It was reported that the concentration of antibodies against the oligomeric forms of A β (1-42) in particular declined with age and advanced Alzheimer's disease (Britschgi *et al.*, Neuroprotective natural antibodies to assemblies of amyloidogenic peptides decrease with normal aging and advancing Alzheimer's disease. *Proc. Natl. Acad. Sci. U S A.*, 2009, 106(29):12145-50).

[0008] It is thus useful to develop biologics that arrest or slow down the progression of the disease without inducing negative and potentially lethal effects on the human body. The need is particularly evident in view of the increasing longevity of the general population and, with this increase, an associated rise in the number of patients annually diagnosed with Alzheimer's disease. It is also useful to develop diagnostic tools for determining the various stages of disease progression. For treatments, the availability of a means of monitoring A β oligomer levels would also be useful during treatment(s).

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SUMMARY

- [0009] Disclosed herein are pharmaceutical compositions comprising, a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) and G (glycine) at position 7, the cyclic peptide being conjugated to a carrier. The carrier comprises, for example, keyhole limpet hemocyanin (KLH), serum albumin, *Concholepas concholepas* hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or a hepatitis core antigen. The pharmaceutical composition can be mixed with an adjuvant which can be an oil-in-water emulsion.
- [0010] In certain embodiments, the adjuvant can be one or more of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).
- [0011] In certain embodiments, the pharmaceutical composition comprising the cyclic peptide can be formulated as a vaccine. In certain embodiments, the cyclic peptide is conjugated to a carrier such as KLH and the adjuvant is an oil-in-water emulsion.
- [0012] Disclosed herein are also pharmaceutical compositions formulated using polyclonal or monoclonal antibodies or antigen-binding fragments thereof produced by immunizing a subject with a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) and G (glycine) at position 7 and can be conjugated with a carrier. In certain embodiments, the polyclonal antibodies or antigen-binding fragments can have a titer ranging from about 10 to about 400 mean fluorescence intensity (MFI). In certain embodiments, the antigen-binding fragments of the antibodies can be Fab, F(ab')₂, scFv, disulfide linked Fv, or mixtures thereof. The pharmaceutical composition can be suitable for parenteral injection or infusion.
- [0013] The disclosure also provides for methods for the treatment or prophylaxis of Alzheimer's disease in a subject comprising the step of administering to the subject a pharmaceutical composition comprising a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) and G (glycine) at position 7,

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the peptide being conjugated to a carrier, and wherein the peptide/immunogen is formulated with and/or emulsified in an adjuvant.

[0014] Also provided are methods for the treatment or prophylaxis of Alzheimer's disease in a subject comprising the step of administering to the subject a pharmaceutical composition comprising a therapeutically effective amount of antibodies or fragments thereof specific to a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) and G (glycine) at position 7 and can be conjugated with a carrier. The antibodies or antigen-binding portions can be polyclonal or monoclonal. The pharmaceutical composition containing the antibodies can be administered at a dose ranging from about 10 µg to about 200 mg antibodies per kg body weight or at a dose ranging from about 10 µg to about 400 µg antibodies per kg body weight.

[0015] Certain embodiments provide methods of: inducing an immune response against oligomeric Amyloid beta in a subject; reducing the Amyloid beta plaque burden in the brain of a subject; improving the cognitive ability of a subject afflicted with an Amyloid beta-related disease, disorder, or condition; vaccinating a subject against an Amyloid beta-related disease, disorder, or condition; and treatment and prophylaxis of Alzheimer's disease in a subject, wherein the methods comprise administering an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence GSNK (SEQ ID NO: 1), SNKG (SEQ ID NO: 2), GSNKG (SEQ ID NO: 3), CSNKG (SEQ ID NO: 4), CGSNKGC (SEQ ID NO: 5), CGNSKGG (SEQ ID NO: 6) and/or CCGSNKGC (SEQ ID NO: 7). In certain embodiments, the cyclic peptide is cyclized by a peptide bond, e.g., by an amide bond. In certain embodiments, the Amyloid beta-related disease is Alzheimer's disease. In certain embodiments, the cyclic peptide is conjugated to a carrier. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, Concholepas concholepas hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH). In certain embodiments, the pharmaceutical composition further comprises an adjuvant. In certain embodiments, the adjuvant is an oil-in-water emulsion. In certain embodiments, the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-

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MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

[0016] Certain embodiments provide methods of inducing an immune response against oligomeric Amyloid beta in a subject. Such methods comprise administering to the subject an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond. In certain embodiments, the immune response against oligomeric Amyloid beta is greater than any immune response against non-oligomeric forms of Amyloid beta in the subject. For example, in certain embodiments, the immune response against oligomeric Amyloid beta is greater than the immune response against monomeric Amyloid beta in the subject. For example, in certain embodiments, the immune response against oligomeric Amyloid beta is at least 3-fold, at least 5-fold, or at least 8-fold greater than the immune response against monomeric Amyloid beta. In certain embodiments, the immune response is selected from the group consisting of an innate response, a humoral response, an antibody response, and a combination of two or more of said immune responses. In certain embodiments, the immune response is an antibody response.

[0017] Certain embodiments provide methods of reducing the Amyloid beta plaque burden in the brain of a subject. Such methods comprise administering to a subject in need of treatment an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond. In certain embodiments, administration of the pharmaceutical composition reduces the amount of plaque by at least about 0.1% to at least about 0.3% over a period of 12 months. In certain embodiments, the reduction in plaque burden is measured in the hippocampus, the parahippocampal cortex, the cerebral cortex, or the amygdala. In certain embodiments, the reduction in plaque burden is measured in the hippocampus, the parahippocampal cortex, or the amygdala.

[0018] Certain embodiments provide methods of improving the cognitive ability of a subject afflicted with an Amyloid beta-related disease, disorder, or condition. Such methods comprise administering to the subject an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond. Certain embodiments provide method of vaccinating a subject against an Amyloid beta-related disease, disorder, or condition, wherein the subject mounts an immune response

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against amyloid beta oligomers. Such methods also comprise administering to a subject in need thereof an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond. In certain of these embodiments, the Amyloid beta-related disease is Alzheimer's disease.

[0019] Certain embodiments provide methods for treatment or prophylaxis of Alzheimer's disease in a subject comprising administering to the subject a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond.

[0020] In any of the methods of administering to the subject a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, the cyclic peptide can be conjugated to a carrier. The carrier can comprise keyhole limpet hemocyanin (KLH), serum albumin, Concholepas concholepas hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH).

[0021] In any of the methods of administering to the subject a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, the pharmaceutical composition further comprises an adjuvant. The adjuvant can be an oil-in-water emulsion. The adjuvant can also be selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

[0022] In any of the above methods, the pharmaceutical composition can be administered by intramuscular injection, intradermal injection, intraperitoneal injection, subcutaneous injection, intravenous injection or infusion, transdermally, oral administration, mucosal administration, intranasal administration, pulmonary administration, parenteral injection or infusion, or

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intrathecal injection or infusion. In any of the above methods, the subject is a mammal. In any of the above methods, the mammal is a human.

[0023] Certain embodiments provide methods of producing a vaccine against an Amyloid beta-related disease, disorder, or condition comprising conjugating a carrier to a cyclic peptide comprising an amino acid sequence GSNK (SEQ ID NO: 1), SNKG (SEQ ID NO: 2), GSNKG (SEQ ID NO: 3), CSNKG (SEQ ID NO: 4), CGSNKGC (SEQ ID NO: 5), CGNSKGG (SEQ ID NO: 6) and/or CCGSNKGC (SEQ ID NO: 7) to form a pharmaceutical composition. In certain embodiments, the cyclic peptide is cyclized by a peptide bond, e.g., by an amide bond. Certain embodiments provide methods of producing a vaccine against an Amyloid beta-related disease, disorder, or condition comprising mixing a pharmaceutical composition comprising a cyclic peptide that comprises an amino acid sequence GSNK (SEQ ID NO: 1), SNKG (SEQ ID NO: 2), GSNKG (SEQ ID NO: 3), CSNKG (SEQ ID NO: 4), CGSNKGC (SEQ ID NO: 5), CGNSKGG (SEQ ID NO: 6) and/or CCGSNKGC (SEQ ID NO: 7) with an adjuvant. In certain embodiments, the cyclic peptide is cyclized by a peptide bond, e.g., by an amide bond. Certain embodiments provide methods of producing a vaccine against an Amyloid beta-related disease, disorder, or condition comprising conjugating a carrier to a cyclic peptide that comprises the amino acid sequence GSNK (SEQ ID NO: 1), SNKG (SEQ ID NO: 2), GSNKG (SEQ ID NO: 3), CSNKG (SEQ ID NO: 4), CGSNKGC (SEQ ID NO: 5), CGNSKGG (SEQ ID NO: 6) and/or CCGSNKGC (SEQ ID NO: 7) to form a pharmaceutical composition, and mixing the pharmaceutical composition with an adjuvant. In certain embodiments, the cyclic peptide is cyclized by a peptide bond, e.g., by an amide bond. In certain embodiments, the Amyloid beta-related disease is Alzheimer's disease. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, Concholepas concholepas hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH). In certain embodiments, the adjuvant is an oil-in-water emulsion. In certain embodiments, the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip-almityl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

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- [0024] Certain embodiments provide for methods of producing a vaccine against an Amyloid beta-related disease, disorder, or condition. The methods can comprise:
- [0025] conjugating a carrier to a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, to form a pharmaceutical composition;
- [0026] mixing a pharmaceutical composition comprising a cyclic peptide that comprises an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, with an adjuvant; or
- [0027] conjugating a carrier to a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, to form a pharmaceutical composition, and mixing the pharmaceutical composition with an adjuvant.
- [0028] In certain embodiments of producing a vaccine against an Amyloid beta-related disease, disorder, or condition, the Amyloid beta-related disease is Alzheimer's disease. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, Concholepas concholepas hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. For example, in certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH). In certain embodiments, the adjuvant is an oil-in-water emulsion. In certain embodiments, the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).
- [0029] The pharmaceutical composition comprising the cyclic peptides or antibodies can be administered by parenteral injection, infusion, intravenous injection or intrathecal injection or infusion. Alternatively, the pharmaceutical composition can be administered subcutaneously, intramuscularly, transdermally or orally.

BRIEF DESCRIPTION OF THE DRAWINGS/FIGURES

- [0030] Figure 1 shows head-to-tail cyclization of CGSNKGG (SEQ ID NO: 6). The free sulfhydryl group can be used for conjugation to a carrier.
- [0031] Figure 2 shows ELISA of CPC cSNK-BSA conjugate (cyclic CGSNKGC (SEQ ID NO: 5) conjugated with BSA, top left-hand panel) versus JPT cSNK-BSA (cyclic CGSNKGG (SEQ ID NO: 6) conjugated with BSA, bottom left-hand panel) and cSNK-KHL conjugate (cyclic CGSNKGG (SEQ ID NO: 6) conjugated with KHL, right-hand panel) in response to murine (m5E3) and humanized (h5E3) monoclonal antibodies.
- [0032] Figure 3 shows binding of murine 5E3 (m5E3) to CPC (upper panel; cyclic CGSNKGC; SEQ ID NO: 5) and JPT (lower panel; cyclic CGSNKGG; SEQ ID NO: 6) synthesized cSNK-BSA conjugate by BIACORE™ analysis. Within the 5 association/dissociation curves, immobilized CPC-cSNK-BSA or JPT-cSNK-BSA with (1) diluent alone, (2) m5E3 prep a, (3) m5E3 prep b, (4) m5E3 prep c, or (5) irrelevant murine IgG1.
- [0033] Figure 4 shows immune response in mice immunized with cSNK-KHL (JPT: cCGSNKGG-KHL; SEQ ID NO: 6). Four mice were immunized subcutaneously every 3 weeks with cSNK-KHL + Emulsigen D adjuvant, and sera collected by tail vein phlebotomy after 9 weeks. Immune response was determined by BIACORE™.
- [0034] Figure 5 is a comparison showing that immunization of mice with cyclic SNK generates antibodies that recognize the cyclic (cCGSNKGG; SEQ ID NO: 6) but not the linear peptide.
- [0035] Figure 6 is a comparison showing that immunization of mice with cyclic SNK (cCGSNKGG; SEQ ID NO: 6) generates antibodies recognizing A β 42 oligomers but not monomers. Immunoreactivity to cyclic SNK peptide and to A β 42 oligomers was determined by BIACORE™. The values are mean binding responses + SEM of antibodies from the four mice.
- [0036] Figure 7A shows that vaccination of mice with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH and adjuvant produces a strong anti-cSNK antibody response.
- [0037] Figure 7B shows a line graph demonstrating a positive correlation between binding of normal human plasma to A β oligomers and cSNK peptide.
- [0038] Figure 8 shows results of cued fear response task testing of APP/PS1 mice vaccinated with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH compared to adjuvant alone.

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- [0039] Figure 9 shows results of contextual fear response task testing of APP/PS1 mice vaccinated with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH compared to adjuvant alone.
- [0040] Figure 10 shows results of novel location recognition task testing of APP/PS1 mice vaccinated with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH compared to adjuvant alone.
- [0041] Figure 11 shows %ThioS plaque area in APP/PS1 mice vaccinated with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH compared to adjuvant alone in the brain regions hippocampus and CA1 parahippocampal cortex.
- [0042] Figure 12 shows %ThioS plaque area in APP/PS1 mice vaccinated with the cyclic SNK (cCGSNKGG; SEQ ID NO: 6) peptide conjugated to KLH compared to adjuvant alone in the brain regions hippocampus, the parahippocampal cortex, and the amygdala.
- [0043] Figure 13 shows median anti-cSNK titer of plasma in immunized animals over the course of vaccination (10 months post primary immunization) from one month post primary immunization (P2) through study (P10) with cyclic SNK (cCGSNKGG; SEQ ID NO:6) peptide conjugated to KLH. Pre-immunization baseline samples and negative control animals immunized with adjuvant alone did not elicit any response to cSNK.

DETAILED DESCRIPTION

Definitions

- [0044] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "a binding molecule," is understood to represent one or more binding molecules. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.
- [0045] Furthermore, "and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. Thus, the term "and/or" as used in a phrase such as "A and/or B" herein is intended to include "A and B," "A or B," "A" (alone), and "B" (alone). Likewise, the term "and/or" as used in a phrase such as "A, B, and/or C" is intended to encompass each of the following embodiments: A, B, and C; A, B, or C; A or C; A or B; B or C; A and C; A and B; B and C; A (alone); B (alone); and C (alone).
- [0046] Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure is related. For example, the Concise Dictionary of Biomedicine and Molecular Biology, Juo, Pei-

Show, 2nd ed., 2002, CRC Press; The Dictionary of Cell and Molecular Biology, 3rd ed., 1999, Academic Press; and the Oxford Dictionary Of Biochemistry And Molecular Biology, Revised, 2000, Oxford University Press, provide one of skill with a general dictionary of many of the terms used in this disclosure.

[0047] Units, prefixes, and symbols are denoted in their Système International de Unites (SI) accepted form. Numeric ranges are inclusive of the numbers defining the range. Unless otherwise indicated, amino acid sequences are written left to right in amino to carboxy orientation. The headings provided herein are not limitations of the various aspects or aspects of the disclosure, which can be had by reference to the specification as a whole. Accordingly, the terms defined immediately below are more fully defined by reference to the specification in its entirety.

[0048] As used herein, the term “non-naturally occurring” substance, composition, entity, and/or any combination of substances, compositions, or entities, or any grammatical variants thereof, is a conditional term that explicitly excludes, but only excludes, those forms of the substance, composition, entity, and/or any combination of substances, compositions, or entities that are well-understood by persons of ordinary skill in the art as being “naturally-occurring,” or that are, or could be at any time, determined or interpreted by a judge or an administrative or judicial body to be, “naturally-occurring.”

[0049] As used herein, the term “conformational epitope” refers to an epitope where the amino acid residues take a particular three-dimensional structure. Antibodies which specifically bind a conformational epitope recognize the spatial arrangement of the amino acid residues of that conformational epitope.

[0050] As used herein, the term “immune response” is broadly defined to encompass all of the mechanisms by which a subject, e.g., a human, defends itself against substances that appear foreign and/or harmful. An immune response is elicited in reaction, either specifically (e.g., an antibody response or a cytotoxic T cell response) or non-specifically (e.g., immune barriers or NK cells) to antigens.

[0051] As used herein, the term “cognitive ability” refers to a subject’s ability to acquire, process, store, remember, retrieve, and act upon information gathered from the environment. For example, in mice, cognitive ability can be measured by tasks including contextual and cued fear conditioning (amygdala-dependent memory) and novel location recognition (hippocampal memory).

[0052] Disclosed herein are pharmaceutical compositions, methods of making them, and methods for the treatment and prophylaxis of Alzheimer’s disease. One aspect provides for an

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immunogen comprising a cyclic peptide having the amino acid sequence CGSNKGG (SEQ ID NO: 6), with the cyclization occurring between C (cysteine) and G (glycine) at position 7 (Figure 1). The cyclic peptide can be conjugated to a carrier. One illustrative example of a useful carrier is KLH. The immunogen can be mixed with an adjuvant such as an oil-in-water emulsion. The cyclic peptide includes a conformational epitope, mimicking a solvent-exposed, antibody accessible knuckle region of oligomeric A β . The conformational epitope can also be part of a cyclic peptide having an amino acid sequence comprising SNK (*i.e.*, serine-asparagine-lysine, or Ser-Asn-Lys) in which the side chain of lysine is oriented into solvent. In certain embodiments, the conformational epitope can be part of a cyclic peptide having an amino acid sequence comprising at least SNK.

[0053] Also disclosed herein are polyclonal or monoclonal antibodies or antigen-binding fragments which are specific to a cyclic peptide having the amino acid sequence CGSNKGG (SEQ ID NO: 6), with the cyclization occurring between C (cysteine) and G (glycine) at position 7.

[0054] Compositions disclosed herein can be used for active or passive immunotherapy of Alzheimer's disease or prophylactic vaccines in populations at risk of developing Alzheimer's disease. Other applications include diagnosis and screening of Alzheimer's disease and monitoring of treatments.

Cyclic peptides

[0055] When A β oligomerizes, a constrained peptide turn forms and takes on a knuckle-like conformation (e.g., see PCT Publications WO2011/106885 and WO2013/071267). In the "turn" region, referred to herein as the "knuckle," region of oligomeric A β , the conformational epitope GSNKG, including the lysine side chain, is exposed to solvent and accessible to antibody binding. This epitope represents a novel target in oligomeric forms of A β . As used herein, the term "A β oligomer", A β O or "oligomeric A β " refers to a form of the A β peptide where the A β are non-covalently aggregated. An A β oligomer can have less than about 200 A β monomers. In one embodiment, an A β oligomer can have less than about 50 A β monomers.

[0056] Image capture of molecular dynamics modeling of a disulfide-linked cyclic peptide comprising residues 25-29 (CGSNKGC; SEQ ID NO: 5) was conducted; non-native cysteines were added for disulfide linkage. This modeling reveals that the side chain of lysine 28 is oriented externally as shown, in contrast to the internally oriented lysine 28 side chain predicted in references Luhrs *et al.*, 3D structure of Alzheimer's amyloid beta(1-42) fibrils, Proc. Natl. Acad. Sci. U S A, 2005, 102(48): 17342-7 and Rauk, A., Why is the amyloid beta peptide of

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Alzheimer's disease neurotoxic? Dalton Trans., 2008(10): 1273-82. The discovery of the outward orientation of the lysine 28 (LYS-28) residue is consistent with the high immunogenicity of this cyclic peptide comprising residues 25-29 (CGSNKGC; SEQ ID NO: 5), whereby the side of lysine is exposed as a large and charged moiety via an ϵ -amino group. The discovery of the outward orientation of the lysine 28 has been verified with authentic A β oligomers also displaying a similar configuration (lysine side-chain orientation in solvent) in an antibody-accessible fashion. The serine 26, asparagine 27 and lysine 28 residues, SNK, located in the knuckle region of A β oligomers are all charged or polar, and have greater immunogenicity than small non-polar amino acids. The cyclic conformation of the SNK residues, located in the knuckle region of A β oligomers, form a novel conformational epitope that is solvent exposed and available for antibody binding. The discovery of this structurally constrained epitope at the surface of A β oligomers has advantageous properties for selective antibody binding.

[0057] In one aspect, the epitope is comprised of strongly polar/charged residues that are solvent-exposed and structurally constrained at the surface of A β oligomers. In another aspect, the structure of the novel conformation-specific epitope is dependent on a relatively-rigid spatial arrangement of the amino acid residues.

[0058] A conformational epitope having a constrained cyclic configuration is not normally present on the molecular surface of APP (amyloid precursor protein), thus limiting the autoimmune recognition of APP. The GSNKG motif of APP that is located at the cell surface of neurons and monocytes is largely unstructured. Thus, conformation-specific antibodies binding to the novel conformational epitope having a constrained cyclic configuration have limited or no recognition of the unstructured GSNKG motif on cell surface APP. Conversely, antibodies recognizing the novel conformational epitope show little or no reaction with monomeric A β .

[0059] Antibodies have been raised that bind to the novel conformational epitope having a constrained cyclic configuration recognize the nonlinear epitope structure in between the subunits in the region of amino acids 25-29 of A β oligomers. The specificity of the antibodies to the novel conformational epitope enables the antibodies to specifically target the oligomeric form of A β and as such, avoid targeting monomeric A β and APP that are known to impact on neuronal and immune function and increase the availability of the antibody for binding, as monomeric A β is present in much larger quantities than oligomeric A β (PCT Publication WO2011/106885).

[0060] Described herein is a peptide comprising a conformational epitope that mimics the knuckle-like epitope in oligomeric A β . In certain embodiments, the conformational epitope has an amino acid sequence comprising SNK. In certain embodiments, the conformational epitope

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can be part of a cyclic peptide having an amino acid sequence comprising at least SNK. The peptide can be a cyclic peptide. The conformational epitope can be part of a peptide bond-linked or disulfide-linked cyclic peptide. The cyclization of the peptide can also occur through any other suitable covalent bonds. Peptides can be cyclic, non-cyclic, branched, linear, or any other suitable form that can give a constrained configuration corresponding to the conformational epitope in oligomeric A β .

[0061] In certain aspects, a cyclic peptide has an amino acid sequence comprising SNK, with the peptide being cyclized through a peptide bond or disulfide bond. In certain aspects, a cyclic peptide has an amino acid sequence comprising SNK, with the peptide being cyclized through a peptide bond. The ring of the cyclic peptide (or the cyclic peptide itself) has 20 or fewer, 15 or fewer, 14 or fewer, 13 or fewer, 12 or fewer, 11 or fewer, 10 or fewer, 9 or fewer, 8 or fewer, 7 or fewer amino acid residues.

[0062] In certain embodiments, the cyclic peptide comprises the sequence CGSNKGG (SEQ ID NO: 6) and is cyclized between C (cysteine) and G (glycine) at position 7 (Figure 1). This head-to-tail peptide bond (amide bond with restricted rotation around carbon) provides high stability while retaining a free sulfhydryl group on the cysteine side chain for conjugation to other entities such as carriers/adjuvants (Figure 1). In order to prevent or minimize dimerization, precautions during synthesis can be taken, including high dilution conditions or using fixed supports for synthesis. The stability of the peptide bond can allow for the antigen for immunizations in a broad range of pH and under reducing conditions (e.g. highly reductive intracellular environment of APCs – antigen presenting cells). In certain embodiments, the cyclic peptide is stable *in vitro* and *in vivo* (e.g., proteolytically, and under both acidic and basic conditions), allows a wide range of formulations, routes of administration, and a specific immune response in favor of soluble A β 42 oligomers rather than linear A β . *In vivo*, the peptide is contemplated to be resistant to reduction and linearization, especially during phagocytosis (internalization and processing through the endocytic pathway of APCs via thiol reductases, e.g., GILT) and general reductive environment of cells. If the cyclic peptide (cCGSNKGG; SEQ ID NO: 6) can be retained through the endocytic pathway of APCs, the probability of presentation to CD4+ lymphocytes can be increased by avoiding degradation of enzymes implicated in this process such as Cathepsin C (dipeptidyl aminopeptidase I), Cathepsin D or asparaginyl endopeptidases.

[0063] In certain embodiments, a conformational epitope-containing peptide can include a glycine residue located at either end of the SNK epitope sequence. The peptide can include

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glycine residues at both ends of the SNK epitope sequence. The glycine residue(s) can have limited or no contribution to the immunogenicity of the conformational epitope and can relieve some steric tension inherent in the cyclization of the peptide. The peptide can include a glycine following lysine closer to the C-terminus and a cysteine closer to the N-terminus of the sequence. The conformational epitope can further include a cysteine followed by a native glycine on the N-terminal and a native glycine and a second glycine on the C-terminal end. The conformational epitope can further include an N-terminal acetylated cysteine, followed by an additional cysteine and a native glycine and a cysteine at the C-terminal end.

[0064] Conformational epitope-containing peptides can comprise any standard (or natural) amino acids, non-standard amino acids, and/or amino acid analogues. Standard amino acids also include selenocysteine and pyrrolysine.

[0065] Non-standard amino acids can be naturally occurring or non-naturally occurring. Non-standard amino acids include any amino acid that can be incorporated into a polypeptide or result from modification of a natural amino acid. Several naturally occurring non-standard amino acids are known in the art, such as 4-hydroxyproline, 5-hydroxylysine, 3-methylhistidine, N-acetylserine, etc. In certain embodiments, an amino acid can be an L-amino acid or a D-amino acid. Further, an amino acid can be subject to any suitable modification, such as methylation, acetylation and/or phosphorylation.

[0066] Alteration can comprise replacing one or more amino acid residue(s) with a non-naturally occurring or non-standard amino acid, modifying one or more amino acid residue into a non-naturally occurring or non-standard form, or inserting one or more non-naturally occurring or non-standard amino acid into the sequence. Non-standard amino acids and amino acid analogues can be incorporated into a peptide during synthesis or by modification or replacement of a natural amino acid after synthesis of a peptide.

[0067] An amino acid can be replaced by another amino acid on the basis of their structure and the general chemical characteristics of their R groups (side-chains). For example, an aliphatic amino acid can be replaced by another aliphatic amino acid; a hydroxyl or sulfur-containing amino acid can be replaced by another hydroxyl or sulfur-containing amino acid; a cyclic amino acid can be replaced by another cyclic amino acid; an aromatic amino acid can be replaced by another aromatic amino acid, a basic amino acid can be replaced by another basic amino acid; an acid amino acid can be replaced by another acid amino acid, etc. Alterations can comprise modifying an L-amino acid into, or replacing it with, a D-amino acid.

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- [0068] In certain embodiments, the conformational epitope comprises an amino acid sequence of SNK in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least SNK.
- [0069] In certain embodiments, the conformational epitope comprises an amino acid sequence of GSNK (SEQ ID NO: 1) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least GSNK.
- [0070] In certain embodiments, the conformational epitope comprises an amino acid sequence of SNKG (SEQ ID NO: 2) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least SNKG.
- [0071] In certain embodiments, the conformational epitope comprises an amino acid sequence of GSNKG (SEQ ID NO: 3) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least GSNKG.
- [0072] In certain embodiments, the conformational epitope comprises an amino acid sequence of CSNKG (SEQ ID NO: 4) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least CSNKG.
- [0073] In certain embodiments, the conformational epitope comprises an amino acid sequence of CGSNKGC (SEQ ID NO: 5) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least CGSNKGC.
- [0074] In certain embodiments, the conformational epitope comprises an amino acid sequence of CGSNKGG (SEQ ID NO: 6) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least CGSNKGG.
- [0075] In certain embodiments, the conformational epitope comprises an acetylated amino acid sequence of CCGSNKGC (SEQ ID NO: 7) in a cyclic constrained configuration or in a cyclic peptide. In one aspect, the conformational epitope comprises an amino acid sequence of at least CCGSNKGC.

Table 1 Peptide Sequences

SEQ ID NO.	Amino Acid Sequence
1	GSNK
2	SNKG
3	GSNKG

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SEQ ID NO.	Amino Acid Sequence
4	CSNKG
5	CGSNKGC
6	CGSNKGG
7	CCGSNKGC

[0076] The conformational epitope of the present antigenic peptide can comprise amino acid residues corresponding to residues 25 to 29 of oligomeric A β (1-40) or oligomeric A β (1-42).

[0077] The conformational epitope can comprise polar/charged amino acid residues that structurally constrained corresponding to the solvent-exposed amino acid residues located at the surface of A β oligomers.

[0078] In certain embodiments, an SNK cyclic peptide can be conjugated to a carrier. In certain aspects, a pharmaceutical composition comprising a cyclic SNK peptide conjugated to a carrier can enhance immunity when administered to a subject as compared to the unconjugated cyclic SNK peptide. In certain embodiments, a pharmaceutical composition comprising a cyclic SNK peptide conjugated to a carrier can elicit T-cell immunity when administered to a subject. In another embodiment, a pharmaceutical composition comprising a cyclic SNK peptide conjugated to a carrier can exhibit an increased *in vivo* half-life when administered to a subject as compared to an unconjugated cyclic SNK peptide.

[0079] Non-limiting examples of carriers include keyhole limpet hemocyanin (KLH), serum albumin (such as bovine serum albumin, BSA), *Concholepas concholepas* hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, diphtheria toxoid, *Neisseria meningitidis* outer membrane complex, *Hemophilus influenza* protein D, hepatitis core antigen, or any combination thereof.

[0080] A carrier can be conjugated to a cyclic SNK peptide of the disclosure by methods known in the art. See, for instance, Pichichero ME, *Hum Vaccine Immunother.* 12:2502-2523 (2013). In certain embodiments, a carrier molecule is conjugated to a free sulfhydryl group on cysteine. A cyclic peptide can be conjugated to a carrier such as KLH using techniques well-known in the art such as EDC conjugation (carboxyl and amine cross linking), maleimide conjugation (sulfhydryl cross linking) or glutaraldehyde conjugation (amine-to-amine cross linking) (www.piercenet.com/method/antibody-production-immunogen-preparation, Pierce Protein Biology Products, Thermo Scientific).

[0081] In certain embodiments, a pharmaceutical composition comprises a cyclic SNK peptide conjugated to a carrier. In certain embodiments, the pharmaceutical composition also comprises an adjuvant. An adjuvant can be added to enhance or modify the immunogenicity of the cyclic SNK peptide. Most antigens activate B cells using activated T helper (Th) cells, primarily Th1 and Th2 cells. The magnitude and type of Th response to a vaccine can be modulated by the choice of adjuvants. See, for instance, Petrovsky *et al.* Vaccine adjuvants: Current state and future trends. *Immunology and Cell Biology* 82:488-496 (2004). In certain embodiments, the pharmaceutical composition elicits immune responses biased towards a Th2 response. For example, the present vaccine can induce predominantly a humoral Th2 response with little or no cellular Th1 response.

[0082] A particulate adjuvant, a non-particulate adjuvant, or combinations thereof can be incorporated into the composition. Particulate adjuvants exist as microparticles or nanoparticles. Cyclic peptides or antibodies can be incorporated or associated with the microparticles. Aluminum salts, water-in-oil emulsions, oil-in-water emulsions, immune stimulating complexes, liposomes, and nano- and microparticles are illustrative examples of particulate adjuvants. The non-particulate adjuvants are generally immunomodulators and they are generally used in conjunction with particulate adjuvants. Muramyl dipeptide (an adjuvant-active component of a peptidoglycan extracted from Mycobacteria), non-ionic block copolymers, Saponins (a complex mixture of triterpenoids extracted from the bark of the Quillaja saponaria tree), Lipid A (a disaccharide of glucosamine with two phosphate groups and five or six fatty acid chains generally C12 to C16 in length), cytokines, carbohydrate polymers, derivatized polysaccharides, and bacterial toxins such as cholera toxin and E. coli labile toxin (LT) are other examples of non-particulate adjuvants. (Petrovsky *et al.* Vaccine adjuvants: Current state and future trends. *Immunology and Cell Biology* 82:488-496 (2004)).

[0083] Some of the adjuvants are a combination of non-particulate immunomodulators and particulate materials, which could impart depot effect to the adjuvant formulation. For example, FCA combines the immunomodulatory properties of Mycobacterium tuberculosis components along with the short-term depot effect of oil emulsions.

[0084] Non-limiting examples of adjuvants include EMULSIGEN[®], EMULSIGEN[®]-D, EMULSIGEN[®]-P (MVP Technologies, Omaha, Nebraska) alum, aluminum phosphate, aluminum hydroxide, MF59 (4.3% w/v squalene, 0.5% w/v polysorbate 80 (Tween 80), 0.5% w/v sorbitan trioleate (Span 85)), CpG-containing nucleic acid (Bode *et al.*, *Expert Rev Vaccine* 10(4):499-511 (2011)), QS21 (saponin adjuvant), MPL (Monophosphoryl Lipid A), 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS (see, e.g., Sjolander *et al.* (1998) J.

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Leukocyte Biol. 64:713; WO90/03184; WO96/11711; WO 00/48630; WO98/36772; WO00/41720; WO06/134423 and WO07/026190), LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, Quil A, interleukins, Freund's, N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (CGP 11637, referred to as nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (CGP 19835A, referred to as MTP-PE), and RIBI, which contains three components extracted from bacteria, monophosphoryl lipid A, trehalose dimycolate and cell wall skeleton (MPL+TDM+CWS) in a 2% squalene/Tween 80 emulsion.

[0085] EMULSIGEN[®]-D and EMULSIGEN[®]-P are proprietary oil-in-water emulsions that are free of animal ingredients. MVP Technologies, Omaha, Nebraska. Both adjuvants can be mixed directly with antigens without further processing.

[0086] The design of a cyclic peptide can be crucial because differences in cyclization can affect its stability, manufacture, and/or the conformation of the epitope produced which can dictate whether a particular immune response is generated. There are at least nine general methods to synthesize cyclic peptides, *i.e.*, head-to-tail, side-chain-to-side-chain, head-to-side-chain, side-chain-to-tail, backbone-to-backbone, head-to-backbone, side-chain-to-backbone, backbone-to-side-chain, and backbone-to-tail. Head-to-tail cycles are usually formed by amide bond formation. Side-chain-to-side-chain cycles are most often synthesized via Cys-Cys or amide bond formation. Cys-Cys cyclization results from the formation of disulfide bridges between cysteine residues of the peptide. Synthetically, this is the most straightforward and frequently used peptide cyclization method according to JPT Peptide Technologies GmbH (world wide web at: www.jpt.com/support/contact/).

[0087] In certain embodiments, a cyclic peptide of a sequence disclosed herein is head-to-tail cyclized with a peptide bond. A head-to-tail peptide bond (such as an amide bond with restricted rotation around carbon) can provide higher stability as compared to a disulfide bond, while at the same time retaining a free sulfhydryl group on the cysteine side chain that can be utilized, *e.g.*, for conjugation to carriers and/or adjuvants. In certain embodiments precautions can be taken during synthesis to prevent dimerization, including high dilution conditions and/or using fixed supports for synthesis. It is contemplated that the stability of the peptide bond provides antigen for immunization in a broad range of pH and under reducing conditions (*e.g.*, the highly reductive intracellular environment of APCs).

[0088] In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at

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position 1 and the G (glycine) at position 7 by a peptide bond. This cyclic peptide can maintain stability while retaining a free sulfhydryl group on the cysteine side chain for conjugation to carriers and/or adjuvants.

Pharmaceutical Compositions

[0089] In certain embodiments, cyclic peptides or antibodies (monoclonal or polyclonal) produced by immunization with the cyclic peptides can be formulated as a pharmaceutical composition for vaccination (*i.e.*, as a vaccine) or for other treatment, prevention, etc. of certain diseases, disorders, or conditions as discussed herein including Alzheimer's disease, for example, as an injectable, liquid solution, emulsions or powder. After conjugation, the cyclic peptides or antibodies (monoclonal or polyclonal) produced by immunization with the cyclic peptides can be mixed with physiologically acceptable carrier solutions which are physiologically compatible; these carrier solutions can include water, saline, dextrose, glycerol, ethanol or any combinations of the foregoing.

[0090] In certain embodiments, pharmaceutical compositions for vaccination can also contain substances, such as wetting or emulsifying agents or pH buffering agents, to further enhance their effectiveness. The pharmaceutical compositions can be administered by injection subcutaneously, intravenously, intramuscularly or intrathecally. Alternatively, the pharmaceutical compositions can be formulated for delivery to mucosal surfaces, such as the eye, nasal passages or respiratory passages. Other modes of administration include the use of suppositories. For suppositories, binders and carriers can include, for example, polyalkylene glycols and triglycerides, while for oral formulations can include normally employed excipients, such as pharmaceutical grades of saccharides, cellulose and magnesium carbonate. The methods for preparing parenterally administrable compositions are well known to those skilled in the art and are described in detail. Bai, J. Neuroimmunol. 80: 65-75, 1997. Warren, J. Neurol. Sci. 152: 31-38, 1997. Tonegawa, J. Exp. Med. 186: 507-515, 1997. When administering the compositions by injection, the administration can be by continuous infusion or by single or multiple bolus injections.

[0091] In certain embodiments, pharmaceutical compositions can be prepared as injectables, either as liquid solutions or suspensions, or as solid forms which are suitable for solution or suspension in liquid vehicles prior to injection. Pharmaceutical compositions can also be prepared in solid form, emulsified or the active ingredient encapsulated in liposome vehicles or other particulate carriers used for sustained delivery. For example, a pharmaceutical composition can be in the form of an oil emulsion, water-in-oil emulsion, water-in-oil-in-water emulsion,

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site-specific emulsion, long-residence emulsion, sticky emulsion, microemulsion, nanoemulsion, liposome, microparticle, microsphere, nanosphere, nanoparticle and various natural or synthetic polymers, such as nonresorbable impermeable polymers such as ethylenevinyl acetate copolymers and HYTREL[®] copolymers, swellable polymers such as hydrogels, or resorbable polymers such as collagen and certain polyacids or polyesters such as those used to make resorbable sutures, that allow for sustained release of the vaccine.

[0092] By way of example, in certain embodiments, intravenously injectable preparations can contain a pharmaceutical composition distributed in a physiologically compatible medium. Suitable medium for the present pharmaceutical compositions can be sterile water for injection (WFI) with or without isotonic amounts of sodium chloride. For example, diluents include sterile WFI, sodium chloride solution (see Gahart, B.L. & Nazareno, A.R., *Intravenous Medications: a handbook for nurses and allied health professionals*, p. 516-521, Mosby, 1997).

[0093] In certain embodiments, a pharmaceutical composition can be orally administered, for example, with an inert diluent or an assimilable edible carrier. The pharmaceutical composition (and other ingredients, if desired) can also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the pharmaceutical composition can be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. In certain embodiments, to administer a pharmaceutical composition by other than parenteral administration, it could be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation. Pharmaceutical compositions for oral administration via tablet, capsule or suspension are prepared using adjuvants including sugars, such as lactose, glucose and sucrose; starches such as corn starch and potato starch; cellulose and derivatives thereof, including sodium carboxymethylcellulose, ethylcellulose and cellulose acetates; powdered tragacanth; malt; gelatin; talc; stearic acids; magnesium stearate; calcium sulfate; vegetable oils, such as peanut oils, cotton seed oil, sesame oil, olive oil and corn oil; polyols such as propylene glycol, glycerine, sorbitol, mannitol and polyethylene glycol; agar; alginic acids; water; isotonic saline and phosphate buffer solutions. Wetting agents, lubricants such as sodium lauryl sulfate, stabilizers, tableting agents, anti-oxidants, preservatives, coloring agents and flavoring agents can also be present.

[0094] Aerosol formulations containing the pharmaceutical compositions, for example, for nasal delivery, can also be prepared in which suitable propellant adjuvants are used. Other adjuvants can also be added to the composition regardless of how it is to be administered, for example, anti-microbial agents can be added to the composition to prevent microbial growth over

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prolonged storage periods. Therapeutic compositions are typically sterile and stable under conditions of manufacture and storage.

[0095] In certain embodiments, antibodies or fragments thereof can be linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol (PEG), and dextran. Such vehicles are described, e.g., in U.S. Application No. 09/428,082 and WO 99/25044. The present antibody or fragment thereof can also be glyco-modified (e.g., deglycosylated, etc.) with improved effector function profile.

[0096] For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated can be used in the pharmaceutical composition. Such penetrants are generally known in the art, and include, e.g., for transmucosal administration, bile salts and fusidic acid derivatives. In addition, detergents can be used to facilitate permeation. Transmucosal administration can be through nasal sprays or using suppositories. Sayani, Crit. Rev. Ther. Drug Carrier Syst. 13: 85-184, 1996. For topical, transdermal administration, the agents are formulated into ointments, creams, salves, powders and gels. Transdermal delivery systems can also include, e.g., patches.

[0097] For inhalation, the pharmaceutical compositions can be delivered using any system known in the art, including dry powder aerosols, liquids delivery systems, air jet nebulizers, propellant systems, and the like. Patton, Biotechniques 16: 141-143, 1998. Also useful are products and inhalation delivery systems for polypeptide macromolecules by, e.g., Dura Pharmaceuticals (San Diego, Calif.), Aradigrn (Hayward, Calif.), Aerogen (Santa Clara, Calif.), Inhale Therapeutic Systems (San Carlos, Calif.), and the like. For example, in certain embodiments the pharmaceutical formulation can be administered in the form of an aerosol or mist. For aerosol administration, the formulation can be supplied in finely divided form along with a surfactant and propellant. In another aspect, the device for delivering the formulation to respiratory tissue is an inhaler in which the formulation vaporizes. Other liquid delivery systems include, e.g., air jet nebulizers.

[0098] In certain embodiments, a pharmaceutical composition can be administered to a subject for treating or preventing the onset or reducing the severity or duration of Alzheimer's disease or other Amyloid beta-related diseases, disorders, or conditions. The data obtained from cell culture assays and animal studies can be used in formulating a range of dosage of the pharmaceutical composition for treatment or preventions, such as vaccination (either cyclic peptide or antibodies), for use in humans or other animal species. In one embodiment, the dosage of such compounds lies within a range of circulating concentrations that include the ED₅₀ with little or no toxicity. The dosage can vary within this range depending upon the dosage form

employed and the route of administration utilized. In certain embodiments, the therapeutically effective dose can be estimated initially from cell culture assays. A dose can be formulated in animal models to achieve a circulating plasma concentration range that includes the IC₅₀ (*i.e.*, the concentration of the test compound which achieves a half-maximal inhibition of symptoms) as determined in cell culture. Sonderstrup, Springer, *Sem. Immunopathol.* 25: 35-45, 2003. Nikula *et al.*, *Inhal. Toxicol.* 4(12): 123-53, 2000.

[0099] In certain embodiments, a pharmaceutical composition is formulated to contain an effective amount of the present cyclic peptide or antibody or antigen-binding portion thereof, wherein the amount depends on the subject to be treated and the condition to be treated. In certain embodiments, the pharmaceutical composition is administered at a dose ranging from about 0.01 mg to about 10 g, from about 0.1 mg to about 9 g, from about 1 mg to about 8 g, from about 1 mg to about 7 g, from about 5 mg to about 6 g, from about 10 mg to about 5 g, from about 20 mg to about 1 g, from about 50 mg to about 800 mg, from about 100 mg to about 500 mg, from about 0.01 mg to about 10 g, from about 0.05 µg to about 1.5 mg, from about 10 µg to about 1 mg protein, from about 30 µg to about 500 µg, from about 40 pg to about 300 pg, from about 0.1 µg to about 200 mg, from about 0.1 µg to about 5 µg, from about 5 µg to about 10 µg, from about 10 µg to about 25 µg, from about 25 µg to about 50 µg, from about 50 µg to about 100 µg, from about 100 µg to about 500 µg, from about 500 µg to about 1 mg, from about 1 mg to about 2 mg. The specific dose level for any particular subject depends upon a variety of factors including the activity of the specific peptide, the age, body weight, general health, sex, diet, time of administration, route of administration, and rate of excretion, drug combination and the severity of the particular disease undergoing therapy.

[0100] In certain embodiments, the pharmaceutical composition can be administered to a subject at a dose ranging from about 1 µg to 1 mg/kg body weight, about 10 µg to 800 µg/kg body weight, about 20 µg to 600 µg/kg body weight, about 30 µg to 500 µg/kg body weight, about 10 µg to 400 µg/kg body weight, about 20 µg to 400 µg/kg body weight, about 60 µg to 100 µg/kg body weight, about 10 µg to 200 µg per kg body weight, about 100 µg to 200 µg/kg body weight, about 50 µg/kg body weight, or about 100 µg /kg body weight.

[0101] In certain embodiments, the dose can also range from about 10 mg/kg of body weight to about 5 g/kg body weight, about 5 mg/kg of body weight to about 2 g/kg body weight, about 50 mg/kg of body weight to about 4 g/kg body weight, about 100 mg/kg of body weight to about 3 g/kg body weight, about 0.1 g/kg body weight to about 1 g/kg body weight, about 0.2 g/kg body weight to about 0.8 g/kg body weight, about 0.2 g/kg of body weight to about 4 g/kg body weight, about 10 mg/kg of body weight to about 50 mg/kg body weight, about 0.2 g/kg body

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weight, about 0.4 g/kg body weight, about 0.8 g/kg body weight, about 5 mg/kg body weight to about 500 mg/kg body weight, at least about 10 mg/kg body weight, at least about 15 mg/kg body weight, at least about 20 mg/kg body weight, at least about 25 mg/kg body weight, at least about 30 mg/kg body weight or at least 50 mg/kg body weight, up to about 100 mg/kg body weight, up to about 150 mg/kg body weight, up to about 200 mg/kg body weight, up to about 250 mg/kg body weight, up to about 300 mg/kg body weight, or up to about 400 mg/kg body weight. In other embodiments, the doses of the immunoglobulin can be greater or less.

[0102] Using a mass/volume unit, a pharmaceutical composition can be administered to a subject at a dose ranging from about 0.1 mg/ml to about 2000 mg/ml, or any amount therebetween, for example 0.1, 0.5, 1, 2, 5, 10, 15, 20, 25, 30, 35, 40, 50, 60, 70, 80, 90, 100, 120, 140, 160 180, 200, 250, 500, 750, 1000, 1500, 2000 mg/ml, or any amount therebetween; or from about 1 mg/ml to about 2000 mg/ml, or any amount therebetween, for example, 1.0, 2.0, 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0 60.0, 70.0, 80.0, 90.0, 100, 120, 140, 160 180, 200, 250, 500, 750, 1000, 1500, 2000, mg/ml or any amount therebetween; or from about 10 mg/ml to about 1000 mg/ml or any amount 15 therebetween, for example, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0 60.0, 70.0, 80.0, 90.0, 100, 120, 140, 160 180, 200, 250, 500, 750, 1000 mg/ml, or any amount therebetween; or from about 30 mg/ml to about 1000 mg/ml or any amount therebetween, for example 30.0, 35.0, 40.0, 50.0 60.0, 70.0, 80.0, 90.0, 100, 120, 140, 160 180, 200, 250, 500, 750, 1000 mg/ml.

[0103] The duration of administration can vary. In certain embodiments, for example, it can range from about 10 minutes to about 1 day, from about 30 minutes to about 20 hours, from about 1 hour to about 15 hours, from about 2 hours to about 10 hours, from about 3 hours to about 8 hours, from about 4 hours to about 6 hours, from about 1 day to about 1 week, from about 2 weeks to about 4 weeks, from about 1 month to about 2 months, from about 2 months to about 4 months, from about 4 months to about 6 months, from about 6 months to about 8 months, from about 8 months to about 1 year, from about 1 year to about 2 years, or from about 2 years to about 4 years, or more. The duration of administration can be about 1 month, about 3 months, about 6 months, about 1 year, about 18 months, about 2 years, about 5 years, or about 10 years. In certain embodiments, the treatment can last the remainder of a subject's natural life.

[0104] In certain embodiments, pharmaceutical compositions can be administered in a single dose treatment or in multiple dose treatments on a schedule and over a time period appropriate to the age, weight and condition of the subject, the particular composition used, and the route of administration. In certain embodiments, a single dose of the pharmaceutical composition is administered. In other embodiments, multiple doses are administered. The frequency of

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administration can vary depending on any of a variety of factors, e.g., severity of the symptoms, degree of immunoprotection sought, whether the composition is used for prophylactic or curative purposes, etc. For example, in certain embodiments, a pharmaceutical composition can be administered about once per day, once per month, twice per month, three times per month, about once every other month, every other week (qow), once per week (qw), twice per week (biw), three times per week (tiw), four times per week, five times per week, six times per week, every other day (qod), daily (qd), twice a day (qid), three times a day (tid), about once every 6 months, about once a year, about once every 2 years, or about once every 5 years, etc. The pharmaceutical composition can also be administered in one or more doses per day.

[0105] Effectiveness of the treatment can be assessed during the entire course of administration after a certain time period, e.g., about every 3 months, about every 6 months, about every 9 years, about every year, etc. The administration schedule (dose and frequency) can be adjusted accordingly for any subsequent administrations. U.S. Patent Nos. 8,066,993 and 7,968,293.

[0106] In certain embodiments, the cyclic peptide, vaccine composition, or antibody or antigen-binding portion thereof concentration in the pharmaceutical composition can range from about 0.1% (w/w) to about 30% (w/w), from about 0.5% (w/w) to about 20% (w/w), from about 1% (w/w) to about 15% (w/w), from about 2% (w/w) to about 3% (w/w), or from about 5% (w/w) to about 10% (w/w).

Treatment of Amyloid Beta-Related Conditions

[0107] In certain embodiments, the pharmaceutical compositions have *in vitro* and/or *in vivo* therapeutic, prophylactic, and/or diagnostic utilities. For example, these pharmaceutical composition can be administered to cells in culture, e.g., *in vitro* or *ex vivo*, or to a subject, e.g., *in vivo*, to diagnose or prevent an amyloid beta-related disease, such as Alzheimer's disease, or diseases, disorders, or conditions involving A β , inhibit or delay the onset of the disease, or slow progression of the disease. Amyloid beta-related diseases, disorders, or conditions include, but are not limited to, Alzheimer's disease, Down's syndrome, dementia pugilistica, multiple system atrophy, inclusion body myositis, hereditary cerebral hemorrhage with amyloidosis of the Dutch type, Nieman-Pick disease type C, cerebral β -amyloid angiopathy, dementia associated with cortical basal degeneration, type 2 diabetes, chronic inflammation, malignancy and Familial Mediterranean Fever, multiple myeloma and B-cell dyscrasias, the prion diseases, Creutzfeldt-Jakob disease, Gerstmann-Straussler syndrome, kuru, scrapie, the amyloidosis associated with carpal tunnel syndrome, senile cardiac amyloidosis, familial amyloidotic polyneuropathy, and the amyloidosis associated with endocrine tumors.

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[0108] Alzheimer patients receiving a pharmaceutical composition(s) disclosed herein can be in the early, middle or late stages of the disease progression, with mild, moderate or severe symptoms. In other cases, individuals suspected of beginning to develop Alzheimer's disease or considered at risk of developing this disease can also receive such treatment, so that their progression towards onset of the disease can be halted or reversed, or their risk of developing the disease can be diminished or eliminated. In other words, the anti-Alzheimer treatment can be applied as a method of preventing Alzheimer's disease or inhibiting or delaying the onset and/or progression of the disease in at-risk individuals with no or only suspected symptoms. U.S. Patent No. 8,066,993.

[0109] In certain embodiments, pharmaceutical compositions disclosed herein can be used for prophylaxis and/or treatment of Alzheimer's disease. A pharmaceutically effective amount of a pharmaceutical composition can be administered to an individual diagnosed with Alzheimer's disease. A pharmaceutically effective amount of a pharmaceutical composition can be administered to an individual at risk for developing Alzheimer's disease or to a person with an unknown risk. An effective amount to be administered to the subject can be determined by a physician with consideration of individual differences in age, weight, disease severity, dose and frequency of administration, and individual response to the therapy.

[0110] In certain embodiments, antibody or antigen-binding portion thereof can be administered alone or in combination with another therapeutic agent, e.g., a second monoclonal or polyclonal antibody or antigen-binding portion thereof, or other therapeutic agents to treat Alzheimer's disease.

[0111] In certain embodiments, pharmaceutical compositions can contain cyclic peptides or antibodies together with one or more other active agents. Alternatively, a pharmaceutical composition can be administered consecutively, simultaneously or in combination with one or more other active agents. Non-limiting examples of the active agents that can be used in combination include an acetylcholinesterase inhibitor (such as tacrine, rivastigmine, galantamine or donepezil) or an NMDA receptor antagonist (such as memantine). Other active agents that can be used in combination include those that are useful for treating Alzheimer's disease or related dementias. For example, the cyclic peptides disclosed herein can be co-formulated coadministered and/or sequentially administered with one or more additional antibodies or cyclic peptides that bind other targets.

Antibody production

- [0112] Also provided herein are antibodies produced by immunizing a subject with the present cyclic peptides. In certain embodiments, antibodies or antigen-binding portion thereof, include, but are not limited to, polyclonal antibodies, monoclonal antibodies, chimeric antibodies, humanized antibodies, recombinant antibodies, as well as antigen-binding portions of the foregoing, e.g., Fab, F(ab')₂, Fab', F(ab)', Fv, single chain Fv (scFv), bivalent polypeptides (e.g., scFV-scFV), multivalent polypeptides (e.g., trivalent scFv (tri-scFv)), disulfide linked Fv, Fc, Fd, dAb fragment, and antibody fusion proteins. An antigen-binding portion of an antibody can include a portion of an antibody that specifically binds to a cyclic peptide as described herein. In certain embodiments, antibodies include murine or human antibodies.
- [0113] Conventional methods can be used to prepare the present antibodies including polyclonal antisera or monoclonal antibodies.
- [0114] To produce polyclonal antibodies, a mammal (e.g. a mouse, hamster, or rabbit) can be immunized with an immunogenic form of the epitope which elicits an antibody response in the mammal. Following immunization, antisera can be obtained and polyclonal antibodies can be isolated from the sera.
- [0115] Certain embodiments provide a method for making a hybridoma that expresses an antibody that specifically binds to a cyclic peptide disclosed herein. The method comprises immunizing an animal with a composition that includes a disclosed cyclic peptide; isolating splenocytes from the animal; generating hybridomas from the splenocytes; and selecting a hybridoma that produces an antibody that specifically binds to the cyclic peptide. Kohler and Milstein, *Nature*, 256: 495, 1975. Harlow, E. and Lane, D. *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988.
- [0116] Animals (e.g., mice) can be immunized subcutaneously, intraperitoneally or intravenously with the antigen. After immunization, one or more boosts may or may not be given. The titers of the antibodies in the plasma can be monitored by, e.g., ELISA (enzyme-linked immunosorbent assay), other immunoassay procedures, or flow cytometry. Animals with sufficient titers of the antibodies against the cyclic peptide are used for fusions. Animals may or may not be boosted with the antigen 3 days before sacrifice and removal of the spleen. Animals may or may not be boosted with a different antigen before sacrifice and removal of the spleen. In mice, the splenocytes are isolated and fused with PEG to a mouse myeloma cell line. The resulting hybridomas are then screened for the production of antigen-specific antibodies. Cells are plated, and then incubated in selective medium. Supernatants from individual wells are then

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screened by ELISA for monoclonal antibodies against the antigen. The antibody secreting hybridomas are re-plated, screened again, and if still positive for anti-antigen monoclonal antibodies, can be subcloned by limiting dilution.

[0117] To purify the anti-antigen antibodies, supernatants from the cultured hybridomas can be filtered and concentrated before affinity chromatography with protein A-Sepharose (Pharmacia, Piscataway, N.J.).

[0118] The immunized animal can be any animal that is capable of producing recoverable antibodies when administered an immunogen, such as, but not limited to, mice, rabbits, rats, hamsters, goats, horses, monkeys, baboons, sharks, camels, llamas, and humans. In one aspect, the host is transgenic and produces human antibodies, e.g., a mouse expressing the human immunoglobulin gene segments. U.S. Patent No. 8,236,311; 7,625,559 and 5,770,429. Lonberg *et al.*, Nature 368(6474): 856-859, 1994. Lonberg, N., Handbook of Experimental Pharmacology 113:49-101, 1994. Lonberg, N. and Huszar, D., Intern. Rev. Immunol., 13: 65-93, 1995. Harding, F. and Lonberg, N., Ann. N.Y. Acad. Sci., 764:536-546, 1995.

Polyclonal Antibodies

[0119] Certain embodiments provide polyclonal antibodies or fragments thereof specific to a cyclic peptide. In certain embodiments, the cyclic peptide comprises the sequence CGSNKGG (SEQ ID NO: 6), which is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 by a peptide bond. The fragments of the antibodies can be Fab, F(ab')₂, scFv, disulfide linked Fv, or mixtures thereof.

[0120] Certain embodiments also provide a composition comprising the polyclonal antibodies or fragments thereof for passive immunotherapies.

[0121] In certain embodiments, the antibody titer can range from about 1 to about 1,000, about 5 to about 800, about 10 to about 600, about 10 to about 400, about 1 to about 50, about 50 to about 100, about 50 to about 200, about 100 to about 200, about 200 to about 400, about 400 to about 600, about 600 to about 800, about 800 to about 1,000, about 1,000 to about 2,000, about 2,000 to about 3,000, about 3,000 to about 4,000, about 5,000 to about 10,000, or about 10,000 to about 20,000 mean fluorescence intensity (MFI). The MFI values correlate with the binding intensity on the MAGPLEX[®] immunoassay based on Luminex's XMAP[®] technology. The use of MFI represents only one possible measure of antibody titer which can be determined using enzyme-linked immunosorbent assay (ELISA) or any other suitable method of measuring antibody titer (described below).

[0122] In certain embodiments, the antibody concentration or titer can range from about 100 µg/ml to about 5 mg/ml, about 200 µg/ml to about 4 mg/ml, about 500 µg/ml to about 3 mg/ml, about 1 mg/ml to about 2 mg/ml, about 2 mg/ml to about 3 mg/ml, about 3 mg/ml to about 4 mg/ml, about 4 mg/ml to about 5 mg/ml, about 100 µg/ml to about 200 µg/ml, about 200 µg/ml to about 300 µg/ml, about 300 µg/ml to about 400 µg/ml, about 400 µg/ml to about 500 µg/ml, about 500 µg/ml to about 600 µg/ml, about 600 µg/ml to about 800 µg/ml, or about 800 µg/ml to about 1 mg/ml.

[0123] In certain embodiments, the polyclonal antibodies or fragments thereof can be in the form of a hyperimmune preparation. The hyperimmune preparation can be isolated from normal or disease affected individuals such as those with dementing diseases, disorders, or conditions, e.g., Alzheimer's disease. The hyperimmune preparations can contain pooled immunoglobulins (*i.e.*, antibodies).

[0124] As used herein, the terms "hyperimmune", "hyperimmune preparation" or "hyperimmune composition" refer to a composition enriched with antibodies specific to one or more particular epitopes. This disclosure provides for a hyperimmune preparation enriched with antibodies specific to a conformational epitope of oligomeric Aβ. For example, the hyperimmune preparation is enriched with antibodies specific to a conformational epitope having an amino acid sequence comprising SNK (*i.e.*, Ser-Asn-Lys) which can correspond to the cyclic peptide. The hyperimmune preparation can be enriched with antibodies specific to a conformational epitope having an amino acid sequence comprising at least SNK. The hyperimmune preparation binds with greater affinity to an oligomeric form of Aβ than to a non-oligomeric form of Aβ. The present hyperimmune preparations can contain an enriched population of antibodies specific to one or more of the conformational epitopes disclosed herein.

[0125] Hyperimmune preparations can contain a high titer or concentration of antibodies specific to one or more of the conformational epitopes disclosed herein. The hyperimmune preparation can be prepared from animal plasma or serum, such as human plasma or serum. The animal can be non-immunized, naturally immunized or artificially immunized with the present conformational epitope. For example, plasma is collected from healthy donors. Plasma can be collected from the same species of animal as the subject to which the immunoglobulin preparation will be administered.

[0126] Plasma or serum can be obtained from animals immunized with a peptide containing a conformational epitope having an amino acid sequence comprising SNK via intramuscular, subcutaneous, intraperitoneal, or intraocular injection, with or without an adjuvant. As used herein, animals can include both human, non-human primates as well as other animals, such as,

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horse, sheep, goat, mouse, rabbit, dog, etc. Optionally, booster immunizations can be done and samples of serum are collected and tested for reactivity to the antigen in standard assays (described below). Once the titer of the animal has reached a plateau in terms of antigen reactivity, larger quantities of the antisera can be obtained readily either by periodic bleeding or exsanguinating the non-human animal.

[0127] For human plasma or serum, prospective donor's plasma is screened to determine the concentration of antibodies that are capable of binding to the conformational epitope. The prospective donor plasma can also be screened to determine the concentration of antibodies to A β peptides, oligomeric A β , and/or combinations thereof.

[0128] In certain embodiments, the hyperimmune preparation is intravenous immunoglobulin (IVIG).

Monoclonal Antibodies

[0129] A murine monoclonal antibody (designated 5E3) has been raised against a cyclic peptide, CGSNKGC (SEQ ID NO: 5), and selectively binds with high affinity to the "cSNK" epitope in AD patient brain homogenates and cerebrospinal fluid; but does not react with non-oligomeric species of AP (monomers, fibrillar/plaque AP, or to cell-surface amyloid precursor protein). PCT Publication WO2011/106885.

[0130] The CDRs of the present antibodies or antigen-binding portions thereof can be from a non-human or human source. The framework of the present antibodies or antigen-binding portions thereof can be human, humanized, non-human (e.g., a murine framework modified to decrease antigenicity in humans), or a synthetic framework (e.g., a consensus sequence).

[0131] In certain embodiments, antibodies or antigen-binding portions thereof, contain at least one heavy chain variable region and/or at least one light chain variable region. The heavy chain variable region (or light chain variable region) contains three CDRs and four framework regions (FRs), arranged from amino-terminus to carboxyl-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Kabat, E. A., *et al.* Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242, 1991. Chothia, C. *et al.*, J. Mol. Biol. 196:901-917, 1987.

[0132] In certain embodiments, antibodies or antigen-binding portions thereof can specifically bind to a cyclic peptide of the present application with a dissociation constant (K_D) of less than about 10^{-7} M, less than about 10^{-8} M, less than about 10^{-9} M, less than about 10^{-10} M, less than about 10^{-11} M, or less than about 10^{-12} M. Affinities of the monoclonal antibodies according to the present disclosure can be readily determined using conventional techniques (*see, e.g.,*

Scatchard *et al.* (1949) Ann. N.Y. Acad. Sci. 51:660; and U.S. Patent Nos. 5,283,173, 5,468,614, or the equivalent).

Humanized Antibodies

[0133] Humanized antibodies can be generated by replacing sequences of the variable region that are not directly involved in antigen binding with equivalent sequences from human variable regions. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of variable regions from at least one of a heavy or light chain. Sources of such nucleic acid are well known to those skilled in the art and, for example, can be obtained from a hybridoma producing an antibody against a cyclic peptide of the present disclosure. The recombinant DNA encoding the humanized antibody, or fragment thereof, can then be cloned into an appropriate expression vector.

[0134] Humanized antibodies can have one, two or three (all) CDRs from the non-human species as well as a framework region from a human immunoglobulin molecule.

[0135] In certain embodiments, humanized antibodies can be produced by methods known in the art. For example, once non-human (e.g., murine) antibodies are obtained, variable regions can be sequenced, and the location of the CDRs and framework residues determined. Kabat, E. A., *et al.* (1991) Sequences of Proteins of Immunological Interest, Fifth Edition, U.S. Department of Health and Human Services, NIH Publication No. 91-3242. Chothia, C. *et al.* (1987) J. Mol. Biol., 196:901-917. The light and heavy chain variable regions can, optionally, be ligated to corresponding constant regions. CDR-grafted antibody molecules can be produced by CDR-grafting or CDR substitution. One, two, or all CDRs of an immunoglobulin chain can be replaced. For example, all of the CDRs of a particular antibody can be from at least a portion of a non-human animal (e.g., mouse) or only some of the CDRs can be replaced. It is generally useful to keep only the CDRs required for binding of the antibody to a predetermined antigen (e.g., a conformational epitope of oligomeric A β). Morrison, S. L., 1985, Science, 229:1202-1207. Oi *et al.*, 1986, BioTechniques, 4:214. U.S. Patent Nos. 5,585,089; 5,225,539; 5,693,761 and 5,693,762. EP 519596. Jones *et al.*, 1986, Nature, 321:552-525. Verhoeyan *et al.*, 1988, Science, 239:1534. Beidler *et al.*, 1988, J. Immunol., 141:4053-4060.

[0136] Also contemplated are antibodies or antigen-binding portions containing one, two, or all CDRs of the present antibodies, together with the other regions replaced by sequences from at least one different species including, but not limited to, human, rabbits, sheep, dogs, cats, cows, horses, goats, pigs, monkeys, apes, gorillas, chimpanzees, ducks, geese, chickens, amphibians, reptiles and other animals.

Chimeric Antibodies

[0137] A chimeric antibody is a molecule in which different portions are derived from different animal species. For example, an antibody can contain a variable region derived from a murine antibody and a human immunoglobulin constant region. Chimeric antibodies can be produced by recombinant DNA techniques. Morrison, *et al.*, Proc Natl Acad Sci, 81:6851-6855 (1984). For example, a gene encoding a murine (or other species) monoclonal antibody molecule is digested with restriction enzymes to remove the region encoding the murine Fc, and the equivalent portion of a gene encoding a human Fc constant region is substituted. Chimeric antibodies can also be created by recombinant DNA techniques where DNA encoding murine V regions can be ligated to DNA encoding the human constant regions. Better *et al.*, Science, 1988, 240:1041-1043. Liu *et al.* PNAS, 1987 84:3439-3443. Liu *et al.*, J. Immunol., 1987, 139:3521-3526. Sun *et al.* PNAS, 1987, 84:214-218. Nishimura *et al.*, Canc. Res., 1987, 47:999-1005. Wood *et al.* Nature, 1985, 314:446-449. Shaw *et al.*, J. Natl. Cancer Inst., 1988, 80:1553-1559. International Patent Publication Nos. WO1987002671 and WO 86/01533. European Patent Application Nos. 184, 187; 171,496; 125,023; and 173,494. U.S. Patent No. 4,816,567.

Types of antibodies

[0138] In certain embodiments, antibodies can be full-length antibodies or can include a fragment (or fragments) of the antibody having an antigen-binding portion, including, but not limited to, Fab, F(ab')₂, Fab', F(ab)', Fv, single chain Fv (scFv), bivalent polypeptides, multivalent polypeptides, antibody fusion proteins and conjugates, disulfide linked Fv, Fc, Fd, dAb fragment (e.g., Ward *et al.*, Nature, 341:544-546 (1989)), an isolated CDR, diabodies, triabodies, tetrabodies, linear antibodies, single-chain, bi-specific, multispecific antibodies formed from antibody fragments, or variants and/or mixtures thereof. Single chain antibodies produced by joining antibody fragments using recombinant methods, or a synthetic linker, are also encompassed herein. Bird *et al.* Science, 1988, 242:423-426. Huston *et al.*, Proc. Natl. Acad. Sci. USA, 1988, 85:5879-5883.

[0139] The antibodies or antigen-binding portions can be monospecific, bi-specific or multispecific. Multispecific or bi-specific antibodies or fragments can be specific for different epitopes of one target polypeptide or can contain antigen-binding domains specific for more than one target polypeptides. In certain embodiments, a multispecific antibody or antigen-binding portion comprises at least two different variable domains, wherein each variable domain is

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capable of specifically binding to a separate antigen or to a different epitope on the same antigen.

[0140] In certain embodiments, antibodies can be linked to or co-expressed with another functional molecule, e.g., another peptide or protein. For example, an antibody or fragment thereof can be functionally linked (e.g., by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody or antibody fragment to produce a bi-specific or a multispecific antibody with a second binding specificity. For example, certain embodiments include bi-specific antibodies wherein one arm of an immunoglobulin is specific for a cyclic peptide, and the other arm of the immunoglobulin is specific for a second therapeutic target or is conjugated to a therapeutic moiety. Other embodiments encompass bi-specific antibodies that are specific both for an epitope of a cyclic peptide and a ligand for capture, detection, removal for therapeutic purposes.

[0141] All antibody isotypes are encompassed herein, including IgG (e.g., IgG1, IgG2, IgG3, IgG4), IgM, IgA (IgA1, IgA2), IgD or IgE. The antibodies or antigen-binding portions thereof can be mammalian (e.g., mouse, human, etc.) antibodies or antigen-binding portions thereof. The light chains of the antibody can be of kappa or lambda type.

[0142] Chemically and/or enzymatically treated antibodies or antigen-binding portion are also included in the present application, such as deglycosylation, pegylation, etc. The antibodies or antigen-binding portion can be molecularly manipulated, such as silencing of glycosylation sites (e.g. glycosylated), etc.

Variations of antibodies

[0143] The antibodies or antigen-binding portions can be formed from peptides. The peptides can also include variants, analogs, orthologs, homologs and derivatives of peptides, that exhibit a biological activity, e.g., binding of an antigen. The peptides can also contain one or more analogs of an amino acid (including, for example, non-naturally occurring amino acids, amino acids which only occur naturally in an unrelated biological system, modified amino acids from mammalian systems etc.), peptides with substituted linkages, as well as other modifications known in the art. In certain instances, specific amino acids can be substituted, deleted, silenced or added.

[0144] Certain embodiments include glycosylation variants of the antibodies or antigen-binding portion described herein. These alternations do not have a substantial effect on the peptide's biological properties such as binding activity, but can improve half-life and/or bioavailability. For example, antibodies can have amino acid substitutions in the framework region, such as to

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improve binding to the antigen. In another example, a selected, small number of acceptor framework residues can be replaced by the corresponding donor amino acids. The donor framework can be a mature or germline human antibody framework sequence or a consensus sequence. Guidance concerning how to make phenotypically silent amino acid substitutions is provided in Bowie *et al.*, Science, 247: 1306-1310 (1990). Cunningham *et al.*, Science, 244: 1081-1085 (1989). Ausubel (ed.), Current Protocols in Molecular Biology, John Wiley and Sons, Inc. (1994). T. Maniatis, E. F. Fritsch and J. Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor laboratory, Cold Spring Harbor, N.Y. (1989). Pearson, Methods Mol. Biol. 243:307-31 (1994). Gonnet *et al.*, Science 256:1443-45 (1992).

[0145] In certain embodiments an antibody or antigen-binding portion can be derivatized or linked to another functional molecule. For example, an antibody can be functionally linked (by chemical coupling, genetic fusion, noncovalent interaction, etc.) to one or more other molecular entities, such as another antibody, a detectable agent, a cytotoxic agent, a pharmaceutical agent, a protein or peptide that can mediate association with another molecule (such as a streptavidin core region or a polyhistidine tag), or facilitate uptake across blood brain barrier (e.g. fusion to cholera toxin A subunits), block or interact with receptors, amino acid linkers, signal sequences, immunogenic carriers, or ligands useful in protein purification, such as glutathione-S-transferase, histidine tag, and staphylococcal protein A. One type of derivatized protein is produced by crosslinking two or more proteins (of the same type or of different types). Suitable crosslinkers include those that are heterobifunctional, having two distinct reactive groups separated by an appropriate spacer (e.g., m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (e.g., disuccinimidyl suberate). Useful detectable agents with which a protein can be derivatized (or labeled) include fluorescent compounds, various enzymes, prosthetic groups, luminescent materials, bioluminescent materials, and radioactive materials. Non-limiting, exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, and, phycoerythrin. A protein or antibody can also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, beta-galactosidase, acetylcholinesterase, glucose oxidase and the like. A protein can also be derivatized with a prosthetic group (e.g., streptavidin/biotin and avidin/biotin).

[0146] In certain embodiments, polypeptides can be a functionally active variant of antibodies of antigen-binding portions thereof disclosed herein, e.g., with less than about 30%, about 25%, about 20%, about 15%, about 10%, about 5% or about 1% amino acid residues substituted or deleted but retain essentially the same immunological properties including, but not limited to, binding to a cyclic peptide disclosed herein.

Antibody assays

- [0147] After the host is immunized and the antibodies are produced, the antibodies can be assayed to confirm that they are specific for the antigen of interest and to determine whether they exhibit any cross reactivity with other antigens. One method of conducting such assays is a sera screen assay as described in U.S. Patent Publication No. 2004/0126829.
- [0148] Antibodies against a cyclic peptide of this disclosure can be characterized for binding to the antigen by a variety of known techniques. The techniques can also be used to measure antibody titer or concentration.
- [0149] For example, in an enzyme-linked immunosorbent assay (ELISA), antigens (e.g., conformational peptide-containing peptides) are first immobilized on a solid support (e.g., a plate). The primary antibody is added, which binds specifically to the test antigen coating the well. This primary antibody can be in a serum sample, a plasma sample, etc. A secondary antibody is added, which will bind the primary antibody. This secondary antibody often has an enzyme attached to it. A substrate for this enzyme is then added. Often, this substrate changes color upon reaction with the enzyme. The higher the concentration of the primary antibody present in the serum, the stronger the color change. Often, a spectrometer is used to give quantitative values for color strength. In quantitative ELISA, the optical density (OD) of the sample is compared to a standard curve, which is typically a serial dilution of a known-concentration solution of the target molecule. For example, if a test sample returns an OD of 1.0, the point on the standard curve that gave OD = 1.0 must be of the same analyte concentration as the sample. Lequin R (2005), Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA), Clin. Chem. 51 (12): 2415–8. Engvall E, Perlman P (1971), Enzyme-linked immunosorbent assay (ELISA), Quantitative assay of immunoglobulin G". Immunochemistry, 8 (9): 871–4. Antibody titer can be determined by serial dilutions. A plasma sample containing antibody can be diluted serially. Using an appropriate detection method each dilution is tested for the presence of detectable levels of antibody. The assigned titer value is indicative of the last dilution in which the antibody was detected.
- [0150] Antibodies can also be assayed by *in vitro* multiplex bead-based immunoassays. Multiplex bead-based immunoassays, such as the LUMINEX[®] XMAP[®] technology, allow the measurement of one analyte or simultaneous measurement of multiple analytes using a library of antigen-containing (or epitope-containing) peptides (or proteins) coupled to color-coded beads. Each bead is identified by the unique wavelength it emits when excited by a laser. Quantitation is accomplished by a sandwich assay using a fluorescently labeled detection antibody with

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affinity to the specific analyte captured by the bead-coupled antibody beads. Excitation by a second laser reads the quantity of bound detection antibody. Houser, Brett, Using Bead-Based Multiplexing Immunoassays to Explore Cellular Response to Drugs, Drug Discover and Development, May 9, 2011. The beads that can be used in the LUMINEX[®] XMAP[®] immunoassays include MAGPLEX[®], MICROPLEX[®], LUMAVIDIN[®], SEROMAP[™] microspheres, etc. MAGPLEX[®] microspheres are superparamagnetic microspheres which are internally labeled with fluorescent dyes and contain surface carboxyl groups for covalent attachment of ligands (or biomolecules). Baker *et al.*, Conversion of a Capture ELISA to a LUMINEX[®] XMAP[®] Assay using a Multiplex Antibody Screening Method, J. Vis. Exp., (65), e4084 10.3791/4084, DOI: 10.3791/4084 (2012). Fulton *et al.*, Advanced multiplexed analysis with the FlowMetrix system. Clinical Chemistry, 43, 1749-1756 (1997). Carson *et al.*, Simultaneous quantitation of 15 cytokines using a multiplexed flow cytometric assay, J. Immunol. Methods, 227, 41-52 (1999).

[0151] In certain embodiments, to detect the presence of antibodies to a cyclic peptide in a sample, the cyclic peptides are coupled to MAGPLEX[®] microspheres based on the multiplexing XMAP[®] platform and analyzed on the MAGPIX[®] instrument (Luminex Corporation, Austin, Texas). The sample is then contacted with cyclic peptide-coupled microspheres, and an immunoassay is used to detect and quantify antibodies specific to the cyclic peptide.

[0152] Different peptides as disclosed herein (e.g., A β (1-42), A β (1-40), and cyclic peptides) can be coupled to different sets or regions of MAGPLEX[®] microspheres. Because each of these regions has a unique internal fluorescent dye, the immunoassay is able to discriminate between the antibodies specific to the different peptides.

[0153] BIACORE[™] assay can be used to characterize antibodies by measuring protein-protein interaction and binding affinity based on surface plasmon resonance (SPR). Karlsson *et al.*, Analysis of active antibody concentration, Journal of Immunological Methods, (1993) 166, 75–84. Markey F., Measuring concentration, Biajournal, 1999, 2: 8 – 11. Antibody titers can also be measured by radioimmunoassay.

Conditions treated

[0154] Pharmaceutical compositions of the various embodiments, including cyclic peptides, vaccine compositions and antibodies or antigen-binding portions, have *in vitro* and *in vivo* therapeutic and/or prophylactic utilities. For example, in certain embodiments, pharmaceutical compositions can be administered to cells in culture, e.g., *in vitro* or *ex vivo*, or to a subject, e.g.,

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in vivo, to treat or prevent Alzheimer's disease, inhibit or delay the onset of Alzheimer's disease, or slow progression of the disease.

[0155] In certain embodiments, pharmaceutical compositions can be used for prophylaxis and/or treatment of Alzheimer's disease. A therapeutically effective amount of the present pharmaceutical composition can be administered to an individual diagnosed with Alzheimer's disease. A pharmaceutically effective amount of the present pharmaceutical composition can be administered to an individual at risk for developing Alzheimer's disease.

[0156] Certain embodiments provide methods of prophylaxis or treatment of Alzheimer's Disease in a subject by administering to the subject a pharmaceutically effective amount of the pharmaceutical composition described herein. An effective amount to be administered to the subject can be determined by a physician with consideration of individual differences in age, weight, disease severity, dose and frequency of administration, and individual response to the therapy.

[0157] In certain embodiments, Alzheimer patients receiving the present compositions can be in the early, middle or late stages of the disease progression, with mild, moderate or severe symptoms. In other embodiments, individuals suspected of beginning to develop Alzheimer's disease or considered at risk of developing this disease can also receive such treatment, so that their progression towards onset of the disease can be halted or reversed, or their risk of developing the disease can be diminished or eliminated. In other words, the anti-Alzheimer treatment can be applied as a method of preventing Alzheimer's disease or inhibiting or delaying the onset of the disease in at-risk individuals with no or only suspected symptoms. U.S. Patent No. 8,066,993.

[0158] In certain embodiments, pharmaceutical compositions can be administered alone or in combination with another therapeutic agent, e.g., a second monoclonal or polyclonal antibody or antigen-binding portion, or other therapeutic agents to treat Alzheimer's disease.

[0159] In certain embodiments, the pharmaceutical compositions can contain the antibodies together with one or more other active agents. Alternatively, the pharmaceutical compositions can be administered consecutively, simultaneously or in combination with one or more other active agents. Non-limiting examples of the active agents that can be used in combination with the present compositions include an acetylcholinesterase inhibitor (such as tacrine, rivastigmine, galantamine or donepezil) or an NMDA receptor antagonist (such as memantine). The other active agents that can be used in combination with the present composition include those that are useful for treating Alzheimer's disease or related dementias. For example, the pharmaceutical

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composition can be co-formulated and/or coadministered with one or more additional antibodies that bind other targets.

[0160] Certain embodiments are directed to methods for diagnosing and screening Alzheimer's disease in a subject.

[0161] The level of antibodies specific to oligomeric A β or the present cyclic peptide can be used to determine a subject's susceptibility to Alzheimer's disease. A positive indication that disease is either present or the patient is at risk for developing Alzheimer's disease will be found when there is a decrease over time in the antibody titer. For example, there can be an about 1%, about 2%, about 3%, about 4%, about 5%, about 5% to about 10%, about 10% to about 20%, about 20% to about 30%, about 40% to about 50%, about 50% to about 60%, about 60% to 70%, about 70% to about 80% or about 80% to about 90% decrease in the antibody titer. The percent decrease is measured as a ratio of the titer at time t1 over t2, where t1 and t2, can differ by about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 6 months to about 12 months, about 8 months to about 16 months, about 16 months to about 24 months, or about 20 months to about 30 months. Other ranges are possible and can be determined by one of ordinary skill in the art. In this embodiment, t1 can represent a control sample. Control samples can also be derived from non-immune serum or from individuals who do not show the presence of any antibody reacting with the epitope.

[0162] Certain embodiments provide methods of diagnosing Alzheimer's Disease in a subject comprising the steps of: (a) obtaining a biological sample from the subject; (b) quantifying in the sample the level of antibodies specific to a conformational epitope having an amino acid sequence comprising at least SNK; and (c) comparing the level of the antibodies in step (b) with a control sample. A different level (e.g., a lower level) of the antibodies specific to the conformational epitope in the sample compared to the control sample are indicative of a diagnosis of Alzheimer's disease in the subject.

[0163] Certain embodiments also provide methods of diagnosing Alzheimer's Disease in a subject comprising the steps of: (a) obtaining a biological sample from the subject; (b) quantifying in the sample the level of antibodies specific to one or more of A β (1-40), A β (1-42), and a conformational epitope having an amino acid sequence comprising at least SNK; and (c) comparing the level of the antibodies in step (b) with a control. A different level (e.g., a lower level) of the antibodies specific to one or more of A β (1-40), A β (1-42), and the conformational epitope in the sample compared to the control are indicative of a diagnosis of Alzheimer's disease in the subject.

- [0164] Certain embodiments provide methods of diagnosing Alzheimer's disease in a subject comprising the steps of: (a) obtaining a sample from the subject; and (b) detecting in the sample the level of antibodies specific to a conformational epitope having an amino acid sequence comprising at least SNK, wherein minimal or no antibodies detected in step (b) is indicative of a diagnosis of Alzheimer's disease in the subject.
- [0165] In certain embodiments, a subject demonstrating an absence of antibodies to the conformational epitope is at a higher risk of developing Alzheimer's disease.
- [0166] Certain embodiments provide methods of predicting a subject's risk of developing Alzheimer's disease comprising the steps of: (a) obtaining a biological sample from the subject; (b) quantifying in the sample the level of antibodies specific to a conformational epitope having an amino acid sequence comprising at least SNK; and (c) comparing the level of the antibodies in step (b) with a control sample. A different level (e.g., a lower level) of the antibodies specific to the conformational epitope in the sample compared to the control sample indicates that the subject is at risk of developing Alzheimer's disease.
- [0167] Certain embodiments also provide methods of predicting a subject's risk of developing Alzheimer's disease comprising the steps of: (a) obtaining a biological sample from the subject; (b) quantifying in the sample the level of antibodies specific to one or more of $A\beta(1-40)$, $A\beta(1-42)$, and a conformational epitope having an amino acid sequence comprising at least SNK; and (c) comparing the level of the antibodies in step (b) with a control. A different level (e.g., a lower level) of the antibodies specific to one or more of $A\beta(1-40)$, $A\beta(1-42)$, and the conformational epitope in the sample compared to the control indicates that the subject is at risk of developing Alzheimer's disease.
- [0168] Non-limiting examples of the control sample include a sample from one or more Alzheimer's disease-free subjects; a sample from one or more subjects having Alzheimer's disease; a stored dataset comprising results generated from studies of one or more Alzheimer's disease-free subjects; a stored dataset comprising results generated from studies of one or more subjects having Alzheimer's disease; a sample from the same subject obtained a time period ago where the time period can be about 6 months, about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years about 7 years, about 8 years, about 9 years, or about 10 years ago; a stored dataset comprising results generated from the same subject a time period of about 6 months, about 1 year, about 2 years, about 3 years, about 4 years, about 5 years, about 6 years about 7 years, about 8 years, about 9 years, or about 10 years; or a clinically selected control. Alternatively, the control sample can be a sample containing a minimum concentration of antibodies to the conformational epitope, or a maximum concentration of antibodies to the

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conformational epitope. The biological sample can be plasma, a tissue, cells, biofluid, or combinations thereof. The biofluid can be cerebrospinal fluid (CSF) or blood.

[0169] When applied *in vitro*, detection methods entail analysis of a biological sample of body fluid or tissue or organ sample from a subject. A tissue or organ sample, such as that obtained from a solid or semi-solid tissue or organ, can be digested, extracted or otherwise rendered to a liquid form. A biological sample or samples can be taken from a subject at any appropriate time, including before the subject is diagnosed with, or suspected of having Alzheimer's disease or a related dementia, during a therapeutic regimen for the treatment or amelioration of symptoms of that disease or disorder, after death of the subject (regardless of the cause, or suspected cause). Alternately, a biological sample can include donated body fluid or tissue, such as blood, plasma or platelets when in care of a centralized blood supply organization or institution. In addition, a biological sample can be taken from a healthy subject to act as a control.

[0170] The present methods can be used for the monitoring of Alzheimer's disease progression within a single patient, or monitoring the effectiveness of a therapeutic agent to slow, stabilize, or reverse the progression of Alzheimer's disease.

[0171] In certain embodiments, the level of antibodies detected to the cyclic peptide in a normal healthy population can be compared to the level of antibodies detected to the cyclic peptide in a known population of subjects with Alzheimer's disease to provide an indication of disease progression or to provide an early diagnosis of Alzheimer's disease.

[0172] Certain embodiments provide methods of inducing an immune response against oligomeric Amyloid beta in a subject. The methods comprise administering to the subject an effective amount of a pharmaceutical composition. In certain embodiments, the pharmaceutical composition comprises a cyclic peptide. In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6) and the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide bond, e.g., an amide bond. In certain embodiments, the immune response against oligomeric Amyloid beta is greater than any immune response against non-oligomeric forms of Amyloid beta in said subject. This is in contrast to immune responses induced using other Amyloid peptides, such as linear Amyloid beta peptides, that lack the constrained epitope described herein and thus would not be selective for oligomeric forms of Amyloid beta or in contrast, selective to linear and/or monomeric forms of Amyloid beta over oligomeric forms. Thus, in certain embodiments the immune response against oligomeric Amyloid beta is greater than the immune response against monomeric Amyloid beta. An immune response can be determined, for example, by assaying the serum of a vaccinated subject

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for binding against oligomeric forms of Amyloid beta and/or for binding against oligomer forms of Amyloid beta in comparison to binding against non-oligomeric forms of Amyloid beta. In certain embodiments, the immune response against oligomeric Amyloid beta is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, or greater than the immune response against non-oligomeric Amyloid beta. In certain embodiments, the immune response against oligomeric Amyloid beta is at least 2-fold, at least 3-fold, at least 4-fold, at least 5-fold, at least 6-fold, at least 7-fold, at least 8-fold, at least 9-fold, at least 10-fold, or greater than the immune response against monomeric Amyloid beta. In any of these embodiments, the oligomeric Amyloid beta can be oligomeric A β 42. In any of these embodiments, the monomeric Amyloid beta is monomeric A β 42. In certain embodiments, the immune response can be an innate response, a humoral response, an antibody response, a cellular response, or a combination of two or more such immune responses. For example, in certain embodiments the immune response is an antibody response.

Immune correlates.

[0173] A body of work has accumulated demonstrating that anti-A β antibodies are effective in clearance of amyloid deposits *in vitro* and in amyloid precursor protein transgenic (AAP-Tg) mice. Frenkel D, *et al.*, Nasal vaccination with a proteosome-based adjuvant and glatiramer acetate clears β -amyloid in a mouse model of Alzheimer disease. *J Clin Invest.* 2005 Sep 1; 115(9): 2423–2433. See also Schenk D, *et al.* Immunization with amyloid beta attenuates Alzheimer-disease-like pathology in the PDAPP mouse. *Nature.* 1999;400:173–177; Weiner HL, *et al.* Nasal administration of amyloid beta peptide decreases cerebral amyloid burden in a mouse model of Alzheimer's disease. *Ann. Neurol.* 2000;48:567–579; Morgan D, *et al.* A beta peptide vaccination prevents memory loss in an animal model of Alzheimer's disease. *Nature.* 2000;408:982–985; Janus C, *et al.* A beta peptide immunization reduces behavioral impairment and plaques in a model of Alzheimer's disease. *Nature.* 2000;408:979–982; Dodart JC, *et al.* Immunization reverses memory deficits without reducing brain A β burden in Alzheimer's disease model. *Nat. Neurosci.* 2002;5:452–457; Bard F, *et al.* Peripherally administered antibodies against amyloid beta-peptide enter the central nervous system and reduce pathology in a mouse model of Alzheimer disease. *Nat. Med.* 2000;6:916–919; and DeMattos RB, *et al.* Peripheral anti-A β antibody alters CNS and plasma A β clearance and decreases brain A β burden in a mouse model of Alzheimer's disease. *Proc. Natl. Acad. Sci. U. S. A.* 2001;98:8850–8855.

[0174] Certain embodiments provide methods of reducing the Amyloid beta plaque burden in the brain of a subject. As used herein, "Amyloid beta plaque" refers to all forms of aberrant deposition of Amyloid beta including large aggregates and small associations of a few Amyloid beta peptides and can contain any variation of the Amyloid beta peptide. Illustrative areas of the brain in which Amyloid plaques develop include the hippocampus, parahippocampal cortex, cerebral cortex, and/or the amygdala. In certain embodiments, a method of reducing the Amyloid beta plaque burden in the brain of a subject comprises administering to a subject in need of treatment an effective amount of a pharmaceutical composition comprising a cyclic peptide. In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6) and is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide bond, e.g., an amide bond. In certain embodiments, administration of the pharmaceutical composition can result in plaque reduction of at least about 0.1% to at least about 0.2%, of at least about 0.1% to at least about 0.3%, of at least about 0.1% to at least about 0.4%, of at least about 0.1% to at least about 0.5%, of at least about 0.1% to at least about 0.6%, of at least about 0.1% to at least about 0.7%, of at least about 0.1% to at least about 0.8%, of at least about 0.1% to at least about 0.9%, of at least about 0.1% to at least about 1.0%. In certain embodiments, administration of the pharmaceutical composition can result in plaque reduction of at least about 0.05% to at least about 0.2%, of at least about 0.05% to at least about 0.3%, of at least about 0.05% to at least about 0.4%, of at least about 0.05% to at least about 0.5%, of at least about 0.05% to at least about 0.6%, of at least about 0.05% to at least about 0.7%, of at least about 0.05% to at least about 0.8%, of at least about 0.05% to at least about 0.9%, of at least about 0.05% to at least about 1.0%. In certain embodiments, administration of the pharmaceutical composition can result in plaque reduction of at least about 0.01% to at least about 0.2%, of at least about 0.01% to at least about 0.3%, of at least about 0.01% to at least about 0.4%, of at least about 0.01% to at least about 0.5%, of at least about 0.01% to at least about 0.6%, of at least about 0.01% to at least about 0.7%, of at least about 0.01% to at least about 0.8%, of at least about 0.01% to at least about 0.9%, of at least about 0.01% to at least about 1.0%. In certain embodiments, administration of the pharmaceutical composition can result in plaque reduction of at least about 0.2% to at least about 0.3%, of at least about 0.2% to at least about 0.4%, of at least about 0.2% to at least about 0.5%, of at least about 0.2% to at least about 0.6%, of at least about 0.2% to at least about 0.7%, of at least about 0.2% to at least about 0.8%, of at least about 0.2% to at least about 0.9%, of at least about 0.2% to at least about 1.0%. In certain embodiments, any of these reductions are observed over a period of about 6, 7, 8, 9, 10, 11, or

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12 months. In certain embodiments, any of these reductions are observed at an endpoint of about 6, 7, 8, 9, 10, 11, or 12 months. In certain embodiments, any of these reductions are observed in the hippocampus, parahippocampal cortex, cerebral cortex, and/or the amygdala. In certain embodiments, any of these reductions are observed in the hippocampus, parahippocampal cortex, and/or the amygdala.

[0175] Certain embodiments provide methods of improving the cognitive ability of a subject inflicted with an Amyloid beta-related disease, disorder, or condition. In certain embodiments, the Amyloid beta-related disease is Alzheimer's disease. Certain embodiments of improving the cognitive ability of a subject inflicted with an Amyloid beta-related disease, disorder, or condition, comprise administering to a subject in need of treatment an effective amount of a pharmaceutical composition comprising a cyclic peptide. In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6) and is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide bond, e.g., an amide bond. Methods of determining improvements in the cognitive ability of a subject are widely known in the field of Alzheimer's and Amyloid-related disease research and include a variety of human and animal behavioral models. For example, Puzzo D, et al., *Biochem Pharmacol.* 2014 Apr 15; 88(4): 450–467, and Bryan KJ, et al., CRC Press; 2009, describe various behavioral test used to study cognitive ability in Alzheimer's disease research including fear conditioning, radial arm water maze, Morris water maze, passive-avoidance learning, Y-maze, T-maze, object recognition, and the open field locomotion test.

[0176] Certain embodiments provide methods of vaccinating a subject against an Amyloid beta-related disease, disorder, or condition, wherein the subject mounts an immune response against amyloid beta oligomers. In certain embodiments, the Amyloid beta-related disease is Alzheimer's disease. Certain embodiments vaccinating a subject against an Amyloid beta-related disease, disorder, or condition, comprise administering to a subject in need of treatment an effective amount of a pharmaceutical composition comprising a cyclic peptide. In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6) and is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide bond, e.g., an amide bond.

[0177] In any of the above methods of inducing an immune response, reducing the Amyloid beta plaque burden, improving cognitive ability, vaccinating a subject, or other treatment, prevention, prophylaxis, etc., the cyclic peptide can be conjugated to a carrier. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin,

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Concholepas concholepas hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. In certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH).

[0178] In any of the above methods of inducing an immune response, reducing the Amyloid beta plaque burden, improving cognitive ability, vaccinating a subject, or other treatment, prevention, prophylaxis, etc., the pharmaceutical composition further comprises an adjuvant. In certain embodiments, the adjuvant is an oil-in-water emulsion. In certain embodiments, the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from *Aquilla*, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

[0179] In any of the above methods of inducing an immune response, reducing the Amyloid beta plaque burden, improving cognitive ability, vaccinating a subject, or other treatment, prevention, prophylaxis, etc., the pharmaceutical composition is administered by intramuscular injection, intradermal injection, intraperitoneal injection, subcutaneous injection, intravenous injection or infusion, transdermally, oral administration, mucosal administration, intranasal administration, pulmonary administration, parenteral injection or infusion, or intrathecal injection or infusion.

[0180] Further, in any of the above methods, the subject can be any animal subject as described herein. For example, in certain embodiments the subject is a mammal. In certain embodiments, the mammal is a human.

Vaccines

[0181] Certain embodiments provide methods of producing a vaccine against an Amyloid beta-related disease, disorder, or condition. In certain embodiments, the disease is Alzheimer's disease. In certain embodiments of producing a vaccine against an Amyloid beta-related disease, the method comprises conjugating a carrier to a cyclic peptide to form a pharmaceutical composition. In certain embodiments, the cyclic peptide comprises an amino acid sequence CGSNKGG (SEQ ID NO: 6) and the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide

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bond. In certain embodiments of producing a vaccine against an Amyloid beta-related disease, the method comprises mixing a pharmaceutical composition comprising a cyclic peptide with an adjuvant. In certain embodiments, the cyclic peptide comprises an amino acid sequence CGSNKGG (SEQ ID NO: 6) and the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized via a peptide bond. In certain embodiments of producing a vaccine against an Amyloid beta-related disease, the method comprises conjugating a carrier to a cyclic peptide to form a pharmaceutical composition, and mixing the pharmaceutical composition with an adjuvant. In certain embodiments, the cyclic peptide comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6) and the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7. In certain embodiments, the cyclic peptide is cyclized a peptide bond.

[0182] In certain embodiments of making a vaccine, the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, *Concholepas concholepas* hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen. For example, in certain embodiments, the carrier comprises keyhole limpet hemocyanin (KLH). In certain methods of making a vaccine, the adjuvant is an oil-in-water emulsion. In certain methods of making a vaccine, the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

Kits

[0183] Also provided for are articles of manufacture comprising packaging material and a pharmaceutical composition. The pharmaceutical composition comprises a cyclic peptide or antibodies (monoclonal or polyclonal) produced by immunization with the cyclic peptide. The packaging material can be labeled to indicate that the composition is useful to treat or prevent Alzheimer's disease. The packaging material can be any suitable material generally used to package pharmaceutical agents including, for example, glass, plastic, foil and cardboard.

[0184] In certain embodiments, a kit for diagnosing Alzheimer's disease or related dementias is provided. The kit comprises a composition as described above, along with instructions for use of

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the composition for the diagnosis or screening of Alzheimer's disease. The instructions can include, for example, dose concentrations, dose intervals, preferred administration methods, methods for immunological screening or testing, or the like. The antibody or antigen-binding portion thereof can further be coupled to a detection reagent. Examples of detection reagents include secondary antibodies, such as an anti-human antibody, an anti-mouse antibody, an anti-rabbit antibody or the like. Such secondary antibodies can be coupled with an enzyme that, when provided with a suitable substrate, provides a detectable colorimetric or chemiluminescent reaction. The kit can further comprise reagents for performing the detection reaction, including enzymes such as proteinase K, blocking buffers, homogenization buffers, extraction buffers, dilution buffers or the like.

[0185] Additional components of the kits can include one or more of the following: instructions for use; another therapeutic agent, an agent useful for coupling an antibody to a label or therapeutic agent, other reagents, or other materials for preparing the antibody for administration; pharmaceutically acceptable carriers; and devices or other materials for administration to a subject.

[0186] Instructions for use can include instructions for therapeutic applications, suggested dosages, dose intervals, and/or modes of administration, etc. Other instructions can include instructions on coupling of the cyclic peptide or antibody or its fragment to a label or a therapeutic agent, or for purification of a conjugate, e.g., from unreacted conjugation components.

[0187] The compositions and methods disclosed herein can be used in all vertebrates, *e.g.*, mammals and non-mammals, including human, mice, rats, guinea pigs, hamsters, dogs, cats, cows, horses, goats, sheep, pigs, monkeys, apes, gorillas, chimpanzees, rabbits, ducks, geese, chickens, amphibians, reptiles and other animals. A subject can be a vertebrate, an experimental animal or a transgenic animal. The present compositions and methods can be for veterinary use.

[0188] The following examples of specific aspects are offered for illustrative purposes only, and are not intended to limit the scope of the present disclosure in any way.

EXAMPLES

Example 1 Cyclized CGSNKGG (SEQ ID NO: 6) peptide

[0189] The presence of cSNK epitope in the cyclized CGSNKGG (SEQ ID NO: 6) peptide was confirmed by ELISA (Figure 2), BIACORE (Figure 3), and Western blotting (data not shown).

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All BIACORE™ experiments were performed using a BIACORE™ 3000 instrument. Certified-grade CM5 sensor chips were activated with a mixture containing equimolar amounts of EDC (N-ethyl-N'-(dimethylaminopropyl)) carbodiide and NHS (*N*-hydroxysuccinimide). Synthetic Abeta(1-42) oligomers and the conformationally-constrained Abeta oligomer epitope mimic, cSNK peptide that is conjugated to BSA, were appropriately diluted and covalently coupled to separate flow cells on the sensor chip surface. Residual unreacted sites on the sensor chip surface were then quenched. A reference surface to account for non-specific binding was accordingly generated by immobilizing BSA on an adjacent flow cell.

[0190] To verify the integrity, activity and specificity of the immobilized ligands, the mouse monoclonal antibody (mAb) 5E3 and an IgG1 isotype control were diluted and injected over the sensor chip surface.

[0191] The murine monoclonal antibody, designated 5E3, was raised against a cyclic peptide, cCGSNKGC (SEQ ID NO: 5). 5E3 selectively binds with high affinity to the "cSNK" epitope in AD patient brain homogenates and cerebrospinal fluid, but does not react with non-oligomeric species of A β (monomers, fibrillar/plaque A β , or to cell-surface amyloid precursor protein). PCT/CA2011/000238.

[0192] These assays confirmed that the newly synthesized cSNK (cCGSNKGG; SEQ ID NO: 6) was immune-recognized by 5E3 monoclonal antibody (murine and humanized IgG). Binding to 5E3 was stronger with KLH-cSNK than with BSA-cSNK. It is known that BSA-cSNK peptides have 4-5 cSNK residues attached to each BSA molecule, while KLH peptides are expected to have many more as the KLH molecule is very large (> 400 kDa) and contains many potential binding sites (up to 80 sites available in maleimide activated KLH).

[0193] A pilot immunization study was also performed on 4 mice with the cSNK-KHL conjugate (cCGSNKGG-KHL; SEQ ID NO: 6) and elicited strong immune responses in mice (Figure 4).

[0194] Table 2 shows BIACORE analysis of mouse sera from cCGSNKGC (SEQ ID NO: 5 synthesized disulfide cyclized peptide) immunized mice, normal mouse sera, murine 5E3 A β O specific antibody and commercial A β mAbs (mAb4G8, mAb6E10, mAb2C8) which recognize the N-terminus of A β and a nonspecific IgG control.

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Table 2

Test Article	Binding Response (RU) to immobilized agent				
	Cyclic SNK-BSA	Linear SNK-BSA	Ab40 Monomer	Cyclic SNK/Linear SNK	Cyclic SNK/Monomer
Mouse anti-cSNK Serum	974.2	172.6	29.0	5.6	33.6
Normal Mouse Serum	4.2	0.7	19.9	6.1	0.2
mAb5E3 (cSNK)	364.0	10.7	0.0	34.2	N/A
mAb4G8 (linear AB17-24)	7.9	-2.0	9216.6	-4.0	0.0
mAb6E10 (linear AB1-17)	56.5	2.4	8949.0	23.2	0.0

Test Article	Binding Response (RU) to immobilized agent		
	Ab42 Oligomer Prep	Ab40 Monomer	Oligomer/Monomer
Mouse anti-cSNK Serum	223.6	51.9	4.3
Normal Mouse Serum	237.4	52.6	4.5
mAb5E3 (cSNK)	112.1	14.7	7.6
mAb6E10 (linear AB1-17)	5717.4	2083.6	2.7
mAb2C8 (linear AB1-16)	8.0	28.3	0.3
IgG1 (AB non-specific)	16.1	6.6	2.4

Example 2 Mice immunized with cyclic-Ser-Asn-Lys (cSNK) peptide generated polyclonal antibodies selective for A β oligomers

[0195] Soluble A β oligomers are thought to mediate most synaptotoxic and neurotoxic effects of A β in AD. We have shown that the mouse monoclonal antibody mAb5E3 generated against the structurally constrained tripeptide cSNK specifically recognizes synthetic and native A β oligomers, but not, monomers or amyloid precursor protein, and also neutralizes A β oligomer toxicity. Further studies of vaccination with cSNK in mice were completed to determine the proportion of polyclonal sera reactive to cyclic epitope.

Methods

[0196] *Preparation of A β 42 Monomers and Oligomers.* Recombinant A β 42 peptide (California Peptide, USA) was dissolved in ice-cold hexafluoroisopropanol (HFIP). The HFIP was removed by evaporation overnight and drying in a SpeedVac centrifuge. To prepare monomers, the peptide film was reconstituted in DMSO to 5 mM, diluted further to 100 μ M in dH₂O and used immediately. Oligomers were prepared by diluting the 5 mM DMSO peptide solution in phenol red-free F12 medium to a final concentration of 100 μ M and incubated for 7 days at 4°C.

[0197] *BIACORE™ Analysis.* Using a BIACORE™ 3000 instrument, equal amounts of cyclic and linear SNK, and A β 42 oligomers and monomers were covalently coupled on separate flow cells of two CM5 sensorchips. Bovine serum albumin (BSA) was similarly immobilized to serve as a reference surface. Oligomer-specific mAb5E3, pan-A β 42 antibody (mAb6E10, Covance,

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USA) mouse IgG1 isotype control were diluted and sequentially injected over the surfaces. Binding responses were monitored in real time and the resultant sensorgrams processed.

[0198] *Immunization of mice.* C3H/B6 and APP/PS1 mice (n=4) were injected with 100 µg of cSNK-KLH + 30% Emulsigen-D and repeat dose between 2 and 3 weeks. Serum was collected prior to initial injection (week 0) and once a week for 3 weeks after the 1st injection.

Results

[0199] Cyclized SNK conjugated with KLH is sufficiently immunogenic to vaccinate in mice. Polyclonal antibodies generated against cyclic SNK do not bind to linear SNK (Figure 5). The humoral immune response against cyclic SNK is specific for Aβ42 Oligomers and not monomers (Figure 6).

Example 3 Study of stability of cyclic peptides

[0200] To study the stability of the different cyclic peptides, *in vitro* assays will be conducted in the presence and absence of proteases associated with the endocytic pathway of APCs [e.g. γ-interferon-inducible lysosomal thiol reductase (Arunachalam *et al*, 2000; Maric *et al*, 2001) cysteine proteases cathepsin (Villadangos *et al*, 1999; Nakagawa *et al*, 1999; Lennon-Dumenil *et al*, 2002), and asparaginyl endopeptidase (Chapman, 1998; Watts, 2001)]. Other variations including degradation across pH and formulations will be performed. In a more elaborate experiment, MHC molecules will be purified from immunized animals and MHC II-associated peptides will be analyzed by tandem mass spectrometry (LC/MS/MS) to demonstrate the improved presentation of cSNK.

[0201] To demonstrate stability, an immune response with enhanced activation of professional APCs and antigen-specific T cells upon antigen uptake will be conducted. Cell surface activation markers and cytokines will be examined and correlated to enhanced immune responses. The opportunity to analyze enriched B cells from immunized mice could also provide evidence of generation of selection cyclic antigen-specific of memory B cells with higher ratios of desired presentation subclasses population.

Example 4 Study of cyclic peptide in animal disease models

[0202] Amyloid beta oligomers (ABOs) are increasingly implicated in neurotoxicity, inhibition of synaptic function, memory loss, behavioral changes, and regional spread of AD Lesne *et al*.. A specific amyloid beta protein assembly in the brain impairs memory. Nature.

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2006;440(7082):352-7; Shankar *et al.* Amyloid beta protein dimers isolated directly from Alzheimer's brains impair synaptic plasticity and memory. *Nat Med.* 2008;14(8):837-42; Meyer-Luehmann *et al.* Exogenous induction of cerebral beta-amyloidogenesis is governed by agent and host. *Science.* 2006;313(5794):1781-4; Cleary *et al.* Natural oligomers of the amyloid beta protein specifically disrupt cognitive function. *Nat Neurosci.* 2005;8(1):79-84.

[0203] We have identified a novel immunological epitope unique to ABOs: the discrete, structurally constrained epitope cyclized serine-asparagine-lysine (cSNK). Antibodies raised against this epitope in mice selectively bind to synthetic ABOs with high affinity (Kd ~ 2.03 nM) and to authentic oligomers found in Alzheimer disease patient brain and cerebrospinal fluid Silverman *et al.* An immunological epitope specific for toxic oligomeric Abeta in Alzheimer's disease. *Alzheimers Dement.* 2012;8(4):P625.

[0204] Antibodies targeting cSNK do not react with non-oligomeric species of A β such as monomeric or fibrillar/plaque A β , nor to full-length amyloid precursor protein Silverman *et al.* An immunological epitope specific for toxic oligomeric Abeta in Alzheimer's disease. *Alzheimers Dement.* 2012;8(4):P625. Moreover we have found that anti-cSNK antibodies neutralize the behavioral toxicity of ABOs injected into the intracerebral ventricles of wild-type mice Silverman *et al.* An immunological epitope specific for toxic oligomeric Abeta in Alzheimer's disease. *Alzheimers Dement.* 2012;8(4):P625. The specificity, affinity, and neuroprotective properties of cSNK-targeting antibodies provide a compelling rationale for the cSNK epitope in active immunotherapy of AD.

[0205] In pilot experiments, cSNK was conjugated with an epitope carrier (KLH). An adjuvant (Emulsigen-D) was also used to increase immunogenicity. Mice produced a robust humoral response to cSNK vaccination (Figures 5, 6, 7A and 7B). Mice vaccinated with cSNK epitope produce strong anti-cSNK antibody response after a second injection. Two month old C3H/Black6 mice were injected once with 100 μ g of cSNK-KLH + 30% Emulsigen-D and again after two weeks. Serum was collected prior to initial injection (week 0) and once a week for 3 weeks after the first injection. The second injection occurred between weeks 2 and 3. cSNK-BSA was immobilized on a CM5 sensor chip, and serial dilutions of the sera were sequentially injected over the immobilized surface. Binding is recorded as response units and expressed here as a fold-change over the pre-injection serum binding value. The * indicates a significant difference ($p < 0.05$) between week 0 or 2 and week 3 or 4. cSNK-BSA and synthetic A β 1-42 oligomers were immobilized on separate flow cells on a CM5 sensor chip surface, together with an adjacent BSA reference flow cell. In order to identify a clinically acceptable peptide/adjuvant

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combination that produces an adequate antibody response (Th2) with minimal cellular cytotoxicity (Th1) and results in improvement of behavioral memory, the following tests will be conducted. Safety and efficacy of multiple cSNK vaccine formulations will be tested in mice. Safety and efficacy of GMP formulated cSNK vaccine will also be tested in canine models of dementia.

[0206] The purpose is to seek effective primary prevention of AD through specific immunological neutralization of neurotoxic ABOs. Previous attempts of A β active vaccination have been hindered by unintended side effects and a lack of efficacy, theorized to be the result, in part, of non-specific targeting of multiple A β species Panza *et al.* Is there still any hope for amyloid-based immunotherapy for Alzheimer's disease? Current opinion in psychiatry. 2014;27(2):128-37. The oligomer selectivity of the cSNK epitope is related to its safety profile as a potential therapeutic. Our deliverables will be development of a clinic-ready vaccine formulation for entry into human safety trials of a protective cSNK vaccine for AD in patients with MCI. The cSNK vaccine can slow the progression of AD, and/or be prophylactic.

Study No. 1

[0207] This study will include good manufacturing practice (GMP) vaccine formulation and production, and safety screening/efficacy testing in the APP/PS1 mouse model of Alzheimer's disease. Mice will be tested in 9 arms with 38 mice per arm.

Methods

[0208] *Animal Injections.* To assess the vaccine formulation with optimal protection against ABO toxicity, we will test multiple adjuvants with diverse immune stimulating properties: Alum, Emulsigen-P, Quil-A, and Quil-A + MPL. Table 3 is a summary of the adjuvants and their immune stimulating properties.

Table 3

Adjuvant	Class	Th1/Th2	CTL	Antibody Production	Licensed Vaccines
Alum	Aluminum hydroxide salts	Th1-/Th2+++	No	++	Many (e.g., DTap, HepB, HepA)
Emulsigen-P	Oil and water emulsion with CpG oligodeoxy-	Th1++/Th2++	No	+++	Veterinary

	nucleotide				
Quil-A	Saponin	Th1++/Th2+	Yes	++	Veterinary
Quil-A/MPL	Saponin plus TLR4 ligand MPL	Th1+++/Th2+	Yes	+++	HPV, HepB

[0209] Mice will be treated with saline alone, adjuvant alone, or adjuvant plus GMP grade cSNK-MAP.

[0210] Peptide injections will consist of 100 micrograms of cSNK-MAP. GMP grade cSNK-MAP will be synthesized by JPT Innovative Peptide Solutions (Acton, MA).

[0211] Subcutaneous injections will begin at 1 month of age and mice will receive a boost every month until end stage, at 15 months of age. Serum will be collected prior to treatment and every month thereafter.

[0212] *Behavior.* Mice will be assessed for memory and cognition using the Novel Object Recognition Test, Conditioned Fear Response Test, and a Y-maze test at 11 months of age and at 14 months.

[0213] *A β Pathology.* At end stage, CSF and brains will be collected from the animals. The effect of vaccination on CSF levels of ABOs will be quantified using the Amorfix Aggregated A-beta Assay (A4), the only assay capable of quantifying A β aggregates in an antibody independent method. One hemibrain from each animal will be processed for histochemistry; plaque load will be quantified by ThioS staining of the hippocampus and cortex. Immunohistochemistry will also be performed, with anti- A β mAb4G8 and mAb6E10 (Covance). Corresponding hemi-brains from each mouse will be homogenized and aggregated Abeta levels determined using the Amorfix A4 assay.

[0214] *Safety Screening.* One possible side effect of vaccination could be an increase of cerebral amyloid angiopathy (CAA), as was observed in the AN1792, vaccination with A β 1-42, clinical trial. Ferrer *et al.* Neuropathology and pathogenesis of encephalitis following amyloid beta immunization in Alzheimer's disease. Brain pathology. 2004;14(1):11-20; Gilman *et al.* Clinical effects of Abeta immunization (AN1792) in patients with AD in an interrupted trial. Neurology. 2005;64(9):1553-62. To assess the effect of vaccination with cSNK on vascular deposition of A β , we will calculate mAb6E10-positive vessels in the brains of immunized and control mice by IHC. IHC will also be used to determine the extent of microglial (MHC-II) and astrocyte (GFAP) activation. Furthermore, to quantify the occurrence of microhemorrhaging, hemibrains from will be stained with Prussian blue. Davtyan *et al.* Immunogenicity, efficacy, safety, and mechanism of action of epitope vaccine (Lu AF20513) for Alzheimer's disease: prelude to a

clinical trial. The Journal of neuroscience : the official journal of the Society for Neuroscience. 2013;33(11):4923-34.

- [0215] *Immune response.* The amount of circulating antibodies targeting cyclized SNK, linear SNK, A β monomers, ABO, and Abeta fibrils will be determined by surface plasmon resonance and a custom Magplex microsphere assay. Gibbs *et al.* Normal healthy donors possess plasma IgG reactive to a neutralizing epitope for toxic and amyloid-seeding beta-amyloid oligomers. Alzheimer's & Dementia: The Journal of the Alzheimer's Association. 2013;9(4):P810-P. The immunoglobulin isotype (*i.e.* IgG1, IgG2, IgM) induced by vaccination will be characterized. It is expected that vaccination with clinically approved adjuvants is equally effective at inducing antibody production as compared to harsher adjuvants.

Study No. 2

- [0216] This study will include safety screening-efficacy testing in canine dementia (performed by the CRO InterVivo Solutions). The adjuvant/cSNK-MAP formulation that provides the most protection and least toxicity in mice in specific aim 1 will be advanced and tested in dogs. We anticipate either Alum or EMUSIGEN-P® to have the highest safety rating, and in that case, intend to test both, with an eye on establishing a veterinary market. For the study, we will utilize a blinded parallel group longitudinal design intended to approximate a clinical trial design for Alzheimer's disease. Measures are recorded at baseline and in a longitudinal fashion over a one-year treatment duration.
- [0217] Subjects will consist of aged Beagle dogs (>6 years old) from the VivoCore colony. All subjects will be highly experienced with respect to cognitive testing and we assume that a minimum of 20 dogs will enter the treatment phase of the study, although 12 subjects per group are recommended. Treatments will begin after an initial screening phase, lasting 6 months. Subjects will be immunized subcutaneously in the back of the neck, a booster injection will be administered 2 weeks later, followed by monthly injections.
- [0218] Subjects will be assessed based on their performance in three cognitive measures, *in vivo* imaging including MRI and PET, all performed by InterVivo (e.g., see Araujo et al. Alzheimer's & Dementia (2013) 9(4):P851 and Cotman et al. J Alzheimers Dis. (2008)15(4):685-707). CSF and plasma will be collected from the animals at regular intervals and the CSF levels of Abeta oligomers and tau, and plasma anti-cSNK and A β oligomer titers will be determined as outlined above.

Example 5 Vaccine Study

[0219] Sex matched cohorts of 38 APP/PS1 mice on a C3H/Black6 background were vaccinated subcutaneously with 100 µg of cSNK-KLH in saline monthly beginning at 3 months of age, with 30% Emulsigen-D (an oil and water emulsion with DDA immunostimulant, made by MVP Technologies, Omaha, NE, USA) as an adjuvant. cSNK was synthesized, head-to-tail cyclized, and conjugated to KLH by JPT Peptide Technologies (Acton MA, USA). Control animals were injected with 30% Emulsigen-D in saline. 19 male and 19 female mice were included to account for the possibility of sex differences in treatment response, as APP/PS1 females are known to have higher plaque burden than males (Donkin, J. J. *et al.* ATP-binding cassette transporter A1 mediates the beneficial effects of the liver X receptor agonist GW3965 on object recognition memory and amyloid burden in amyloid precursor protein/presenilin 1 mice. *J Biol Chem* 285, 34144-34154, doi:10.1074/jbc.M110.108100 (2010); Havas, D. *et al.* A longitudinal study of behavioral deficits in an AbetaPP transgenic mouse model of Alzheimer's disease. *J Alzheimer's Dis* 25, 231-243, doi:10.3233/JAD-2011-101866 (2011)). The animals were treated until 12 months of age, and plasma was collected once prior to treatment and at monthly intervals thereafter. The study included a dropout design that has allowed for neuropathological assessments in a subset of four animals from each treatment group at three intermediate time points (6, 8, and 10 months of age), while leaving sufficient mice (N=26) for appropriately powered behavioral and neuropathological assessment at the end of the study period. At 11 months of age the animals were subjected to behavioral testing using conditioned fear response (CFR, both the cued and contextual) task as well as a novel location recognition (NLR) task. At 12 months of age, mice were sacrificed and plasma, CSF and brains harvested. Animals were anesthetized with 20 mg/kg xylazine (Bayer) and 150 mg/kg ketamine (Bimeda-MTC) via i.p. injection. CSF (approximately 15 µL/mouse) is collected from the cisterna magna as described (Hirsch-Reinshagen, V. *et al.* The absence of ABCA1 decreases soluble ApoE levels but does not diminish amyloid deposition in two murine models of Alzheimer disease. *J Biol Chem* 280, 43243-43256, doi:10.1074/jbc.M508781200 (2005); Donkin, J. J. *et al.* ATP-binding cassette transporter A1 mediates the beneficial effects of the liver X receptor agonist GW3965 on object recognition memory and amyloid burden in amyloid precursor protein/presenilin 1 mice. *J Biol Chem* 285, 34144-34154, doi:10.1074/jbc.M110.108100 (2010)), after which animals are transcardially perfused with PBS heparin (0.5 µg/ml) for 5 min followed by tissue collection. Brains are longitudinally bisected, rapidly frozen over dry ice and stored at -80 °C.

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[0220] With all data, the Shapiro-Wilk test was performed to test the data normality. Differences between groups, genders, and role of naive mice were tested using a regression model with categorical covariates (ANOVA) and t-test or Mann-Whitney test when appropriate; we used XLSTAT V2014.6.04 for statistical analysis.

Results:

[0221] Vaccination was well tolerated, only three cSNK-KLH vaccinated and five control mice were lost over the course of the trial. Of the cSNK mice, one died of fight wounds, one was found dead, and one was ill and required sacrifice.

Cued Fear Conditioning (amygdala-dependent memory):

[0222] *Contextual and Cued Fear Conditioning Task.* The contextual and cued fear conditioning task was utilized to assess learning and memory as described in accordance to previously detailed methods (“Methods of Behavior Analysis in Neuroscience, 2nd edition – Ch 2 Cued and Contextual Fear Conditioning for Rodents” by Curzon, Rustay and Browman 2009). The fear conditioning apparatus consisted of two adjoining compartments, one illuminated (20.3 x 15.9 x 21.3 cm) and one darkened (20.3 x 15.9 x 21.3 cm), divided by a guillotine door (Med Associates Inc., St. Albans, VT). The floor of the compartments consisted of steel rods capable of delivering an electric footshock. The electric shock was delivered by a Programmable Animal Shocker (Med Associates Inc.). The device was cleaned with 70% EtOH between the training and context testing session and Virox during the cued testing session to remove all olfactory cues. Prior to all training and testing sessions mice were placed in clean “start cages” to separate them from their cage-mates to avoid affecting the untested cage-mates. Mice were returned to their home cage after training and testing completed.

[0223] *Training.* During the training session, mice were placed in the illuminated compartment and allowed to habituate to the internal testing environment for 120 seconds. After the habituation period, an 80 dB auditory cue was played for 30 seconds. During the last two seconds of the auditory cue, a mild foot shock (0.8 mA) was administered. After shock presentation, mice were left undisturbed in the testing chamber for an inter-trial interval of 60 seconds. Mice were presented with a second identical tone, shock trial. After the second shock presentation, mice were left undisturbed in the testing chamber for 60 seconds.

[0224] *Context Testing.* Twenty-four hours after training, mice were tested for their ability to remember the context in which they received a foot shock. Testing was conducted in as similar a

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manner as possible to the training session. Mice were placed in the illuminated compartment and observed for the presence/absence of freezing response over a period of 5 minutes.

[0225] *Cued Testing.* Four hours post-context testing, mice were tested for their ability to remember the cue presented pre-shock during the training session. Testing was conducted in an environment different from the training and context testing session (darkened compartment, false floor placed over the steel rods, different cleaning solution). Mice were placed in the darkened compartment and allowed to habituate for 120 seconds. After the habituation period, the same 80 dB auditory cue played during the training session was played for 120 seconds. After the tone presentation mice were left undisturbed in the testing chamber for 60 seconds.

[0226] As shown in Figure 8, vaccination with cSNK significantly improved the amount of time the animals stopped moving in the Cued Fear response task.

[0227] Under these testing conditions, no cSNK immunization effect was found for either sex in the Contextual Fear Response or NLR tasks (Figure 9).

Novel Location Recognition (hippocampal memory):

[0228] *Novel Location Recognition (NLR) task.* The NLR task was utilized to assess object location memory. The testing arena consisted of a plastic chamber (Med Associates Inc) located in a dimly lit room. The objects used in this task consisted of one plastic die and one rubber spiky fish. The arena and all objects were cleaned with 70% EtOH between each training and testing sessions to remove all olfactory cues.

[0229] *Habituation phase.* Two days prior to training and testing mice were allowed to freely explore the empty arena. The habituation phase consisted of two, ten minute sessions administered once per day.

[0230] *Training phase.* Twenty-four hours after the second habituation phase, mice were trained on the task. During training, each mouse was placed into the arena containing both objects (plastic die, rubber spiky fish). The objects were placed close (approximately 10 cm) to the two adjacent corners of the arena. Mice were allowed to explore the arena for five minutes before being returned to their home cage. After an ITI of four hours, the testing phase was administered.

[0231] *Testing phase.* The procedure for the testing phase was identical to that of the training phase except the location of one object (spiky fish) was moved to the opposite corner (from the spiky fish) of the arena. For the purposes of the task, object exploration was defined as interaction with the object via sniffing, touching, or rearing.

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Results:

[0232] Under these testing conditions, no cSNK immunization effect was found for either sex in the Contextual FC or NLR tasks. However, in females there is a clear trend toward improvement. For unknown reasons, 4 of the male animals did not participate in the task, never left one corner of the testing box, and as such were excluded from analysis, reducing the number of males included to only 3 adjuvant treated (Figure 10).

Anti-cSNK Titers: (see Example 6 below)

[0233] Baseline anti-cSNK titers were below detection limits for cSNK and adjuvant-alone animals, and remained as such in the adjuvant alone-animals, while anti-cSNK titers were evident 4 weeks after first immunization and were maintained throughout the study (Figure 13). Titre levels increased significantly from P2 to P3, and high levels were maintained (Figure 13).

ThioS plaque staining:

[0234] Plaque load was determined with ThioflavinS staining. Hemi-brains were post fixed in 4% paraformaldehyde. Hemi-brain coronal sections (25-40 μ m) were stained with 1% Thioflavin-S, images of specific brain regions taken at 5x with an epifluorescence microscope, and amyloid plaques quantified using ImagePro software (Oxplow image tiles using 15 ms exposure with 4.9% Fit-C gain).

[0235] Plaque burden increased with age in the APP/PS1 mouse model in both the hippocampus and the CA1 parahippocampal cortex, as expected. Due to low sample number, the effect of immunization on plaque load at early time points could not be established (Figure 11).

[0236] In females, cSNK immunization reduced the ThioS plaque burden in the amygdala. In the hippocampus, where A β pathology originates, a trend of reduction is evident, however the effect of cSNK immunization on plaque in this brain region did not reach significance (Figure 12).

Example 6: Immunization of APP/PS1 mice with KLH-cSNK results in high anti-cSNK antibody titers in plasma

[0237] The Bio-Plex® MAGPIX™ Multi-Plex Reader enables the detection of IgG antibodies in plasma or serum samples by binding to antigens that are covalently coupled to magnetic beads. In order to measure anti-cSNK antibody levels in mouse plasma samples, MapPlex®-C microspheres (Luminex Corporation, Austin, TX) were coupled to BSA-conjugated cyclic

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peptide cSNK (CGSNKGG; SEQ ID NO: 6) to avoid detection of antibodies raised against KLH carrier. The beads contain two classification fluorophores that absorb at 635 nm and emit at two distinct wavelengths. The conjugated beads serve as the solid phase for the binding of anti-cSNK antibodies in solution, followed by binding of a PE-anti-mouse IgG Fc γ labelling antibody that absorbs maximally at 532 nm and emits at a third distinct wavelength. The MAGPIXTM instrument detects the three distinct wavelengths emitted from the particles and uses them to classify the samples and determine the mean fluorescent intensity. To account for background BSA binding, different regions of MapPlex[®]-C microspheres were coupled to BSA alone and are subtracted from the binding signal to BSA-cSNK. Standard curves were generated by 4-parameter plotting the concentrations of 5E3 proteins with correlated mean fluorescent intensity, and the sample concentrations will be calculated based on standard curve values.

Methods

[0238] *Coupling of cyclic peptides to MapPlex[®]-C microspheres.* For the coupling of KLH-BSA or BSA to different regions of MapPlex[®]-C microspheres, 1.25×10^6 microspheres were prepared in 4-morpholineethanesulfonic acid (50 mM, MES buffer), pH 6.0 and activated with the addition of N-hydroxysulfosuccinimide (Sulfo-NHS) and 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride (EDC), both at a concentration of 50 mg/mL in water. The microspheres were incubated at room temperature for 20 minutes, centrifuged, and the resulting pellet was washed twice in MES (100 mM), pH 6.0. The final pellet was re-suspended in MES (100 mM) buffer, pH 6.0 and 10 μ g for each 1x scale coupling reaction to either BSA-cSNK or BSA. After coupling for 2 hours at room temperature or overnight at 2-8 °C in darkness, the reaction was blocked for 30 min at room temperature with phosphate buffered saline (PBS) containing 1% (w/v) bovine serum albumin (BSA) and 0.05% sodium azide, pH 7.4. The coupled microspheres were then washed twice with PBS with Tween 20 (PBS-T) by centrifugation. The coupled microspheres were then re-suspended in storage buffer (PBS, 1% BSA, 0.05% sodium azide, pH 7.4). Each coupled microsphere type (BSA-cSNK or BSA) were counted using a hemocytometer and stored in the dark at 2-8 °C until ready for use.

[0239] *MapPlex[®] multiplex immunoassays.* For each assay, peptide coated microspheres (BSA-cSNK) and BSA coated microspheres diluted in PBS-T to 3000 microspheres/100 μ L/well in 96-well microplates. Thereafter, 50 μ L of diluted mouse plasma samples in PBS, 2.5% mouse serum, pH 7.4 were added to the microspheres, in duplicate. Prior to sample analysis, all mouse plasma samples were diluted to ensure they fell on a standard curve prepared using purified murine 5E3 monoclonal antibody in PBS, 2.5% mouse serum, pH 7.4, between 1.95-4000 ng/ml

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When tested results were grouped, <30% of intra-assay CV was used as the criteria for obtaining an average result from the same sample with different dilution factors. The mouse plasma sample:microsphere mixture was incubated at room temperature on a platform shaker for 60 minutes in the dark. Unbound antibodies and remaining matrix were removed by placing the microplate on the magnetic separator plate for 1 minute and decanting the supernatants by inversion while still attached to magnetic separator plate. The coupled microspheres and complexed antibodies from mouse plasma were washed twice with 100 μ L of PBS-T by magnetic separation as described above, allowing 1 minute between washed. For detection, a volume of 100 μ L per well of R-phycoerythrin labeled anti-mouse IgG (2 μ g/ml) and allowed to incubate at room temperature on a platform shaker for 30 min in the dark. Excess detection antibody was removed by magnetic separation as described, and bound material was washed twice with 100 μ L per well PBS-T, also by magnetic separation as described. The complexed microspheres were then re-suspended in 100 μ L of PBS-T and measured using the MAGPIX® analyzer instrument to determine a Luminex mean fluorescence intensity (MFI) value.

Results:

- [0240] Baseline (pre-dose) samples – A total of 73 samples were tested; 72 had negative fluorescent signals indicating no anti-cSNK antibody binding. 1 subject had equal signal against BSA beads and BSA-cSNK beads, and therefore it had significant non-specific binding and was omitted from further analysis.
- [0241] Control group (adjuvant alone) – From each subject only the first and last sample bleed were tested. They were tested at the minimum dilution of 1 in 40. Only 2 subjects had specific signals that were greater than their non-specific signals (to BSA) and fell in standard curve range. Only one of these subjects had a signal that was high enough that we may want to test at a greater dilution than 1 in 40.
- [0242] Test group (KLH-cSNK vaccinated) – samples were tested at 4 dilutions; 1 in 4000, 1 in 16000, 1 in 64000, and 1 in 256000.
- [0243] In all subjects, there is an increase in titer/antibody concentration from pre-immune to 2 months after immunization and the titer/antibody concentration remained sustained throughout the remainder of the study (Figure 13).
- [0244] The scope of the present disclosure is not limited by what has been specifically shown and described hereinabove. Those skilled in the art will recognize that there are suitable alternatives to the depicted examples of materials, configurations, constructions and dimensions.

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Numerous references, including patents and various publications, are cited and discussed in the above description.

[0245] All references, publications, patents and patent applications cited and discussed in this specification are incorporated herein by reference in their entirety into the specification to the same extent as if each individual reference, publication, patent or patent application was specifically and individually indicated to be incorporated herein by reference. Also incorporated by reference is any supplemental information that was published along with any of the aforementioned references, publications, patents and patent applications. For example, some journal articles are published with supplemental information that is typically available online. Variations, modifications and other implementations of what is described herein will occur to those of ordinary skill in the art without departing from the spirit and scope of the disclosure. While certain embodiments have been shown and described, it will be obvious to those skilled in the art that changes and modifications can be made without departing from the spirit and scope of the disclosure. The matter set forth in the foregoing description and accompanying drawings is offered by way of illustration only and not as a limitation.

WHAT IS CLAIMED IS:

1. A method of inducing an immune response against oligomeric Amyloid beta in a subject, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond.
2. The method of claim 1, wherein the immune response against oligomeric Amyloid beta is greater than any immune response against non-oligomeric forms of Amyloid beta in the subject.
3. The method of claim 1 or claim 2, wherein the immune response against oligomeric Amyloid beta is greater than the immune response against monomeric Amyloid beta in the subject.
4. The method of claim 3, wherein the immune response against oligomeric Amyloid beta is at least 3-fold, at least 5-fold, or at least 8-fold greater than the immune response against monomeric Amyloid beta.
5. The method of any one of claims 1 to 4, wherein the immune response is selected from the group consisting of an innate response, a humoral response, an antibody response, and a combination of two or more of said immune responses.
6. The method of claim 5, wherein the immune response is an antibody response.
7. A method of reducing the Amyloid beta plaque burden in the brain of a subject comprising administering to a subject in need of treatment an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond.
8. The method of claim 7, wherein administration of the pharmaceutical composition reduces the amount of plaque by at least about 0.1% to at least about 0.3% over a period of 12 months.
9. The method of claim 7 or claim 8, wherein the reduction in plaque burden is measured in the hippocampus, the parahippocampal cortex, the cerebral cortex, or the amygdala.
10. The method of claim 9, wherein the reduction in plaque burden is measured in the hippocampus, the parahippocampal cortex, or the amygdala.

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11. A method of improving the cognitive ability of a subject inflicted with an Amyloid beta-related disease, disorder, or condition, comprising administering to the subject an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond.

12. A method of vaccinating a subject against an Amyloid beta-related disease, disorder, or condition, comprising administering to a subject in need thereof an effective amount of a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between the C (cysteine) at position 1 and the G (glycine) at position 7 via a peptide bond, wherein the subject mounts an immune response against amyloid beta oligomers.

13. The method of claim 11 or claim 12, wherein the Amyloid beta-related disease is Alzheimer's disease.

14. A method for treatment or prophylaxis of Alzheimer's disease in a subject comprising administering to the subject a pharmaceutical composition comprising a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond.

15. The method of any one of claims 1 to 14, wherein the cyclic peptide is conjugated to a carrier.

16. The method of claim 15, wherein the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, *Concholepas concholepas* hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen.

17. The method of claim 16, wherein the carrier comprises keyhole limpet hemocyanin (KLH).

18. The method of any one of claims 1 to 17, wherein the pharmaceutical composition further comprises an adjuvant.

19. The method of claim 18, wherein the adjuvant is an oil-in-water emulsion.

20. The method of claim 18, wherein the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts

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from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dip- almitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

21. The method of any one of claims 1 to 20, wherein the pharmaceutical composition is administered by intramuscular injection, intradermal injection, intraperitoneal injection, subcutaneous injection, intravenous injection or infusion, transdermally, oral administration, mucosal administration, intranasal administration, pulmonary administration, parenteral injection or infusion, or intrathecal injection or infusion.

22. The method of any one of claims 1 to 21, wherein the subject is a mammal.

23. The method of claim 22, wherein the mammal is a human.

24. A method of producing a vaccine against an Amyloid beta-related disease, disorder, or condition, comprising:

(a) conjugating a carrier to a cyclic peptide comprising an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, to form a pharmaceutical composition;

(b) mixing a pharmaceutical composition comprising a cyclic peptide that comprises an amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, with an adjuvant; or

(c) conjugating a carrier to a cyclic peptide that comprises the amino acid sequence CGSNKGG (SEQ ID NO: 6), wherein the peptide is cyclized between C (cysteine) at position 1 and G (glycine) at position 7 via a peptide bond, to form a pharmaceutical composition, and mixing the pharmaceutical composition with an adjuvant.

25. The method of claim 24, wherein the Amyloid beta-related disease is Alzheimer's disease.

26. The method of claim 24 or claim 25, wherein the carrier comprises keyhole limpet hemocyanin (KLH), serum albumin, *Concholepas concholepas* hemocyanin (CCH), ovalbumin, an immunoglobulin Fc domain, tetanus toxoid, or hepatitis core antigen.

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27. The method of claim 26, wherein the carrier comprises keyhole limpet hemocyanin (KLH).

28. The method of any one of claims 24 to 27, wherein the adjuvant is an oil-in-water emulsion.

29. The method of any one of claims 24 to 27, wherein the adjuvant is selected from the group consisting of alum, Quil A, MPL (Monophosphoryl Lipid A), MF59, AS04, QS21 (saponin adjuvant), aluminum hydroxide, complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA), aluminum phosphate, sorbitan trioleate, CpG-containing nucleic acid, 3DMPL (3-O-deacylated MPL), extracts from Aquilla, ISCOMS, LT/CT mutants, poly(D,L-lactide-co-glycolide) (PLG) microparticles, interleukins and N-acetyl-muramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-nor-muramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'-dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (MTP-PE).

Head-to-tail cyclization of CGSNKGG

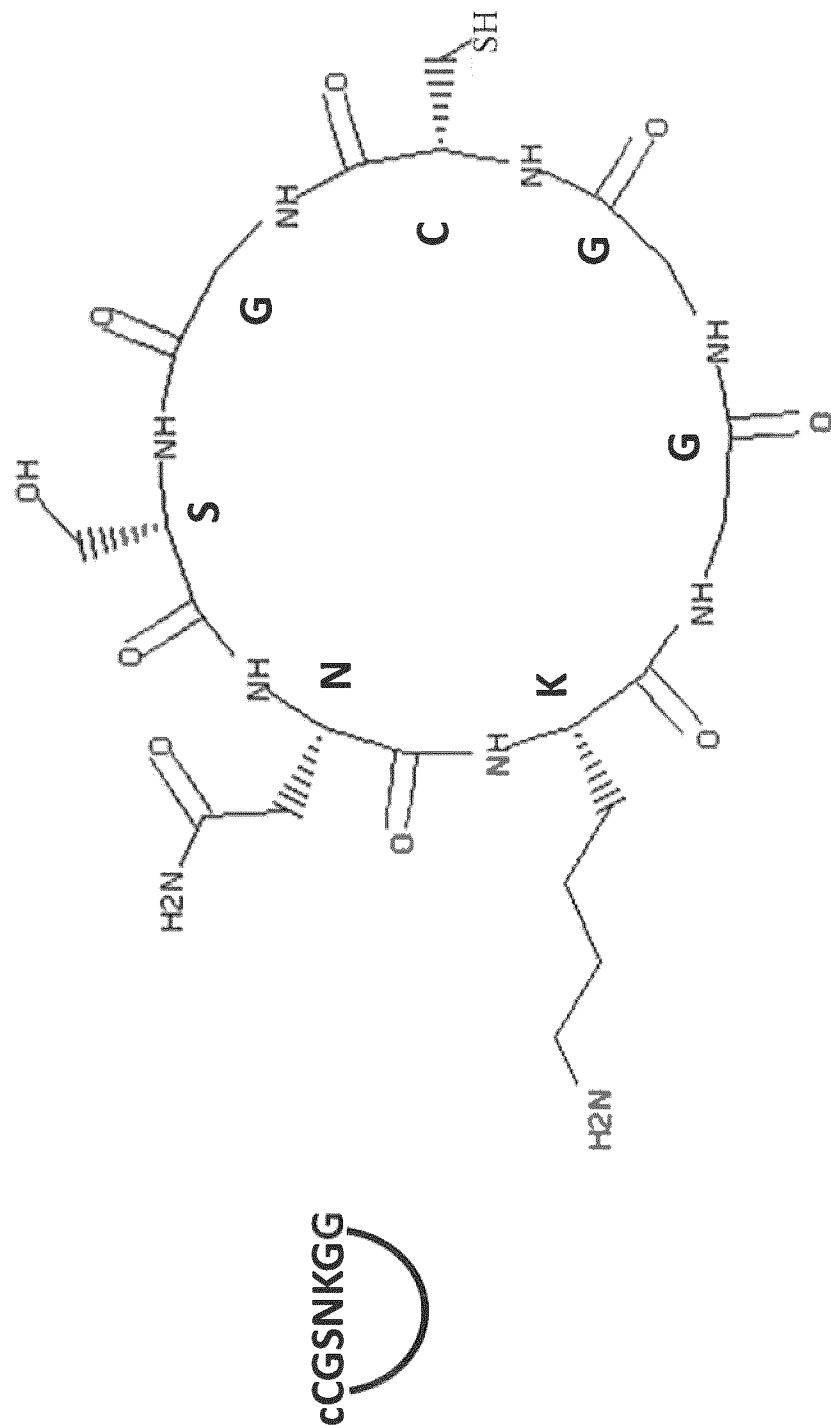


Figure 1

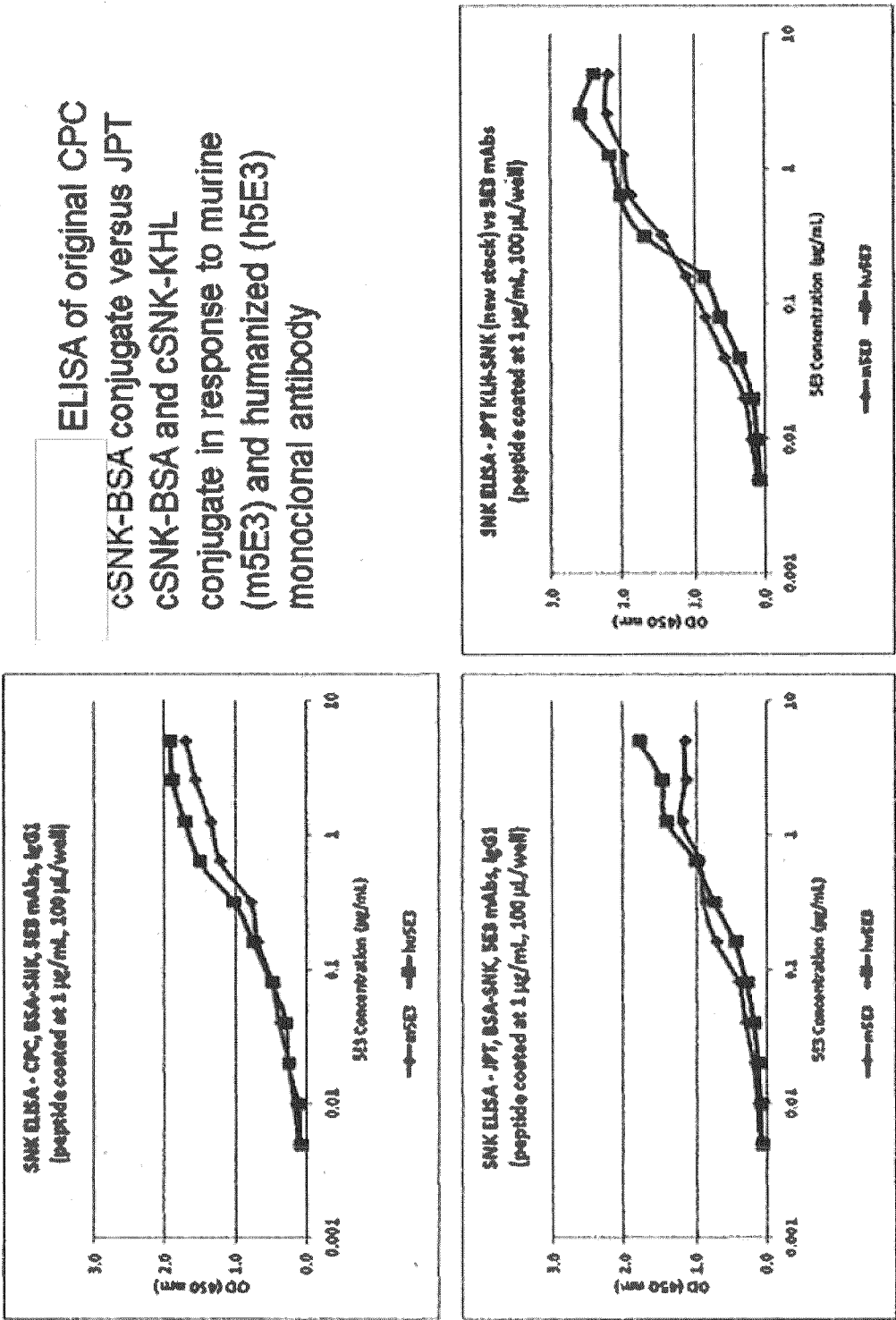
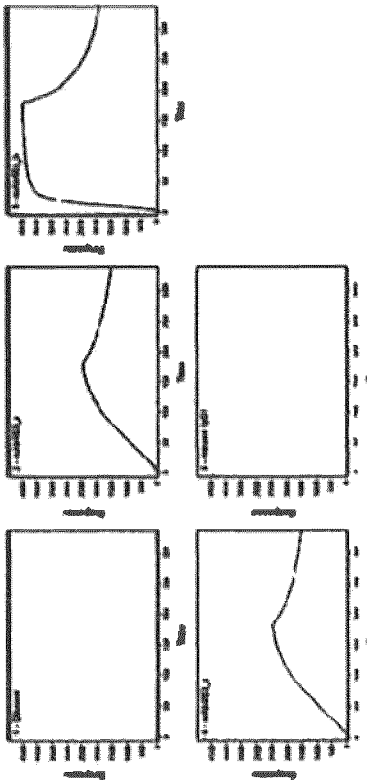
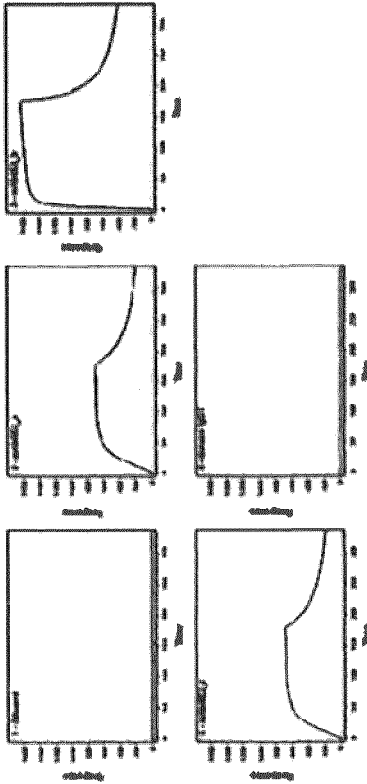


Figure 2

Binding of mAb5E3 to CPC_SNK-BSA



Binding of mAb5E3 to JPT_SNK-BSA



binding of murine 5E3 (mAb5E3) to CPC (upper panel) and JPT (lower panel) synthesized cSNK-BSA conjugate by Biacore analysis. Within the 5 association/dissociation curves; immobilized CPC-cSNK-BSA or JPT-cSNK-BSA with (1) diluent alone, (2) m5E3 prep a, (3) m5E3 prep b, (4) m5E3 prep c, or (5) irrelevant murine IgG1

BSA-SNK, CPC lot CK-050589
BSA-SNK, JPT, Lot 261113Q-01

Figure 3

Immune response in mice immunized with cSNK-KHL (JPT: cCGSNKGG-KHL). Four mice were immunized subcutaneously every 3 weeks with cSNK-KHL (JPT: cCGSNKGG-KHL). + Emulsigen D adjuvant, and sera collected by tail vein phlebotomy after 9 weeks. Immune response was determined by Biacore.

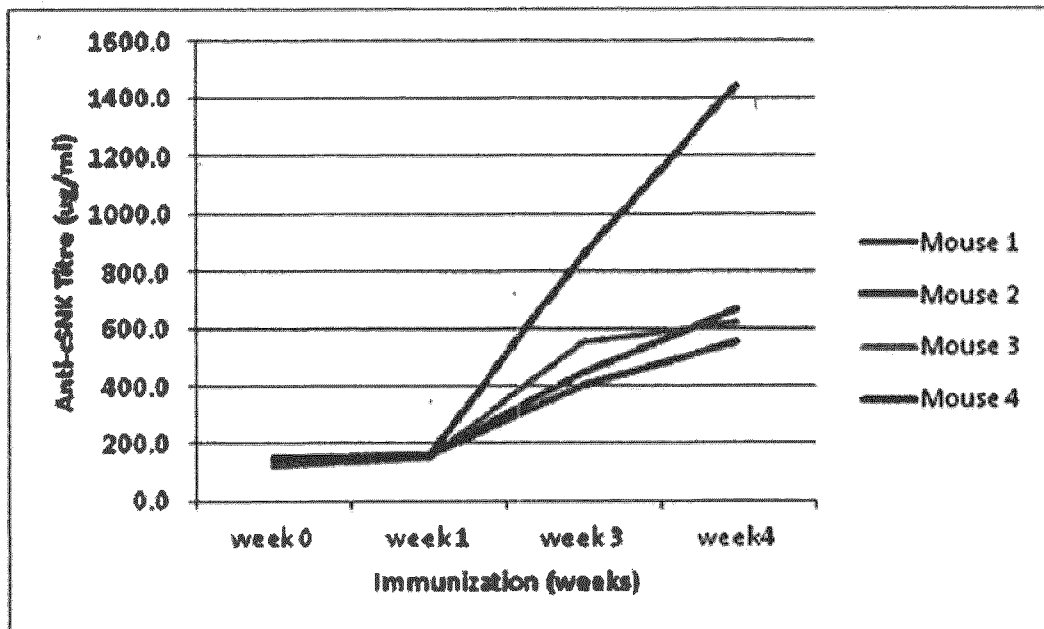


Figure 4

Immunization of mice with cyclic SNK generates antibodies that recognize the cyclic but not the linear tripeptide

Binding to Cyclic and Linear SNK Peptides

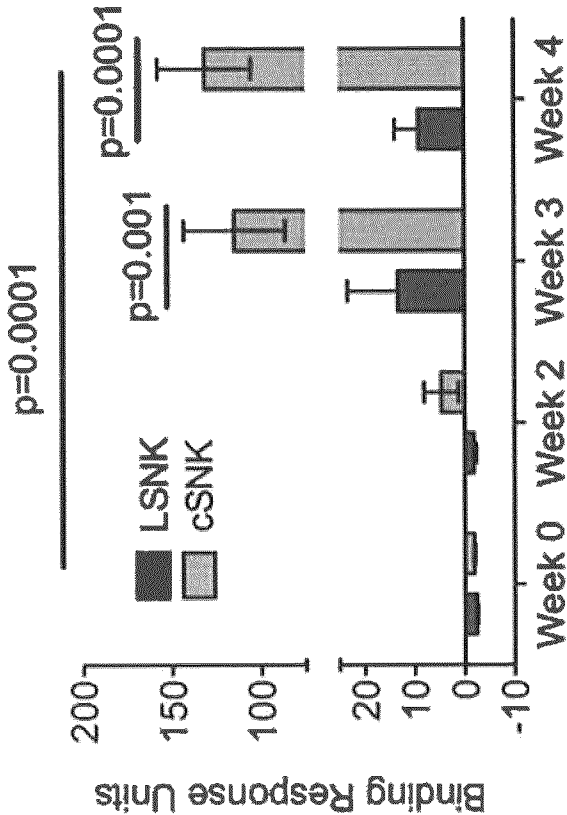


Figure 5

Immunization of Mice with Cyclic SNK Generates Antibodies Recognizing A β 42 Oligomers but not Monomers

Binding to A β 42 Oligomers but not Monomers

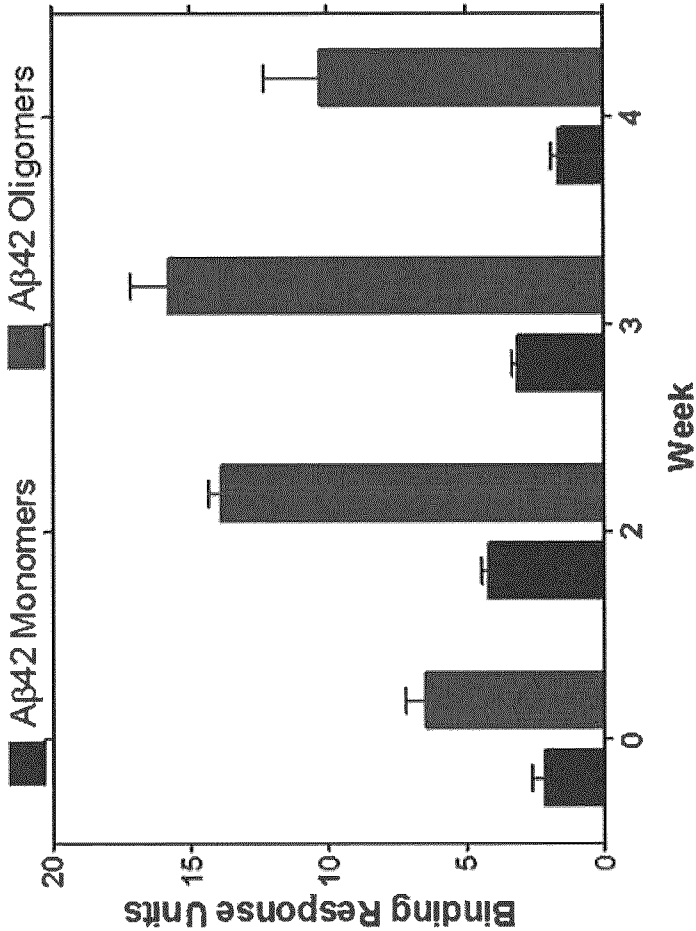


Figure 6

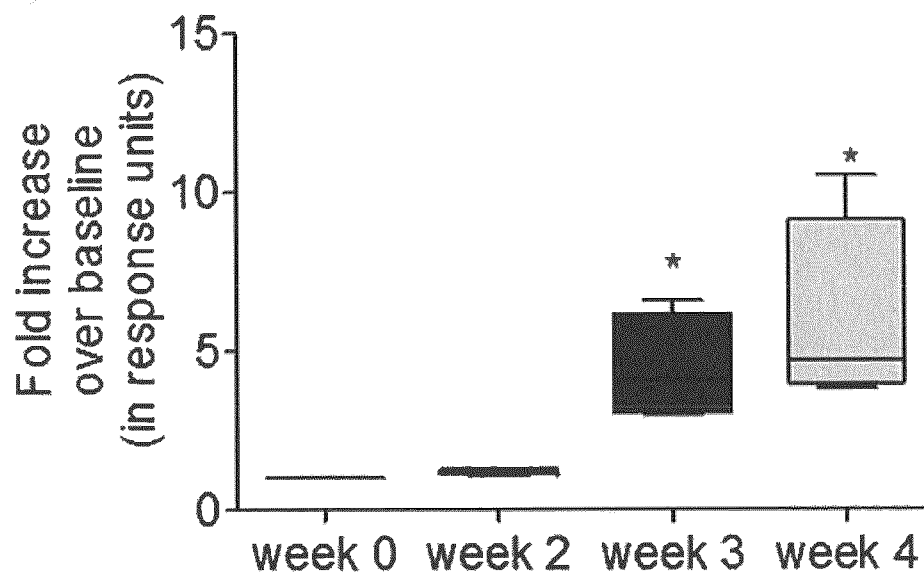


Figure 7A

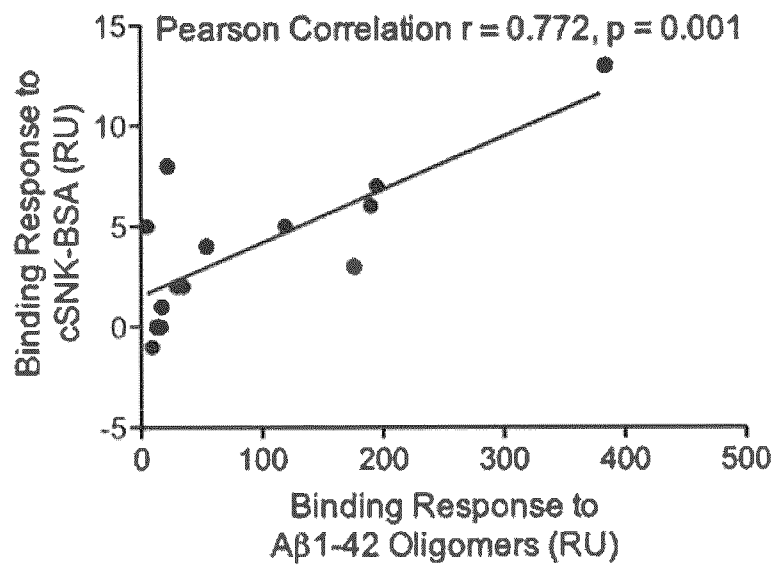


Figure 7B

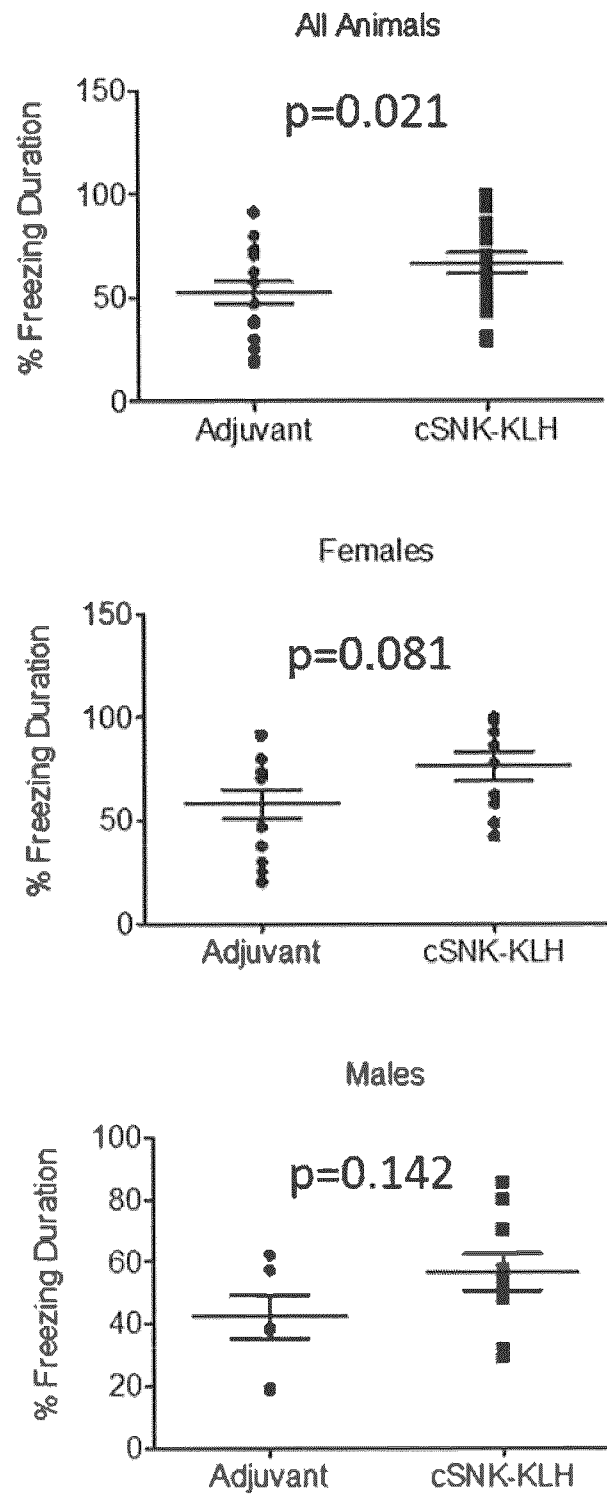


Figure 8

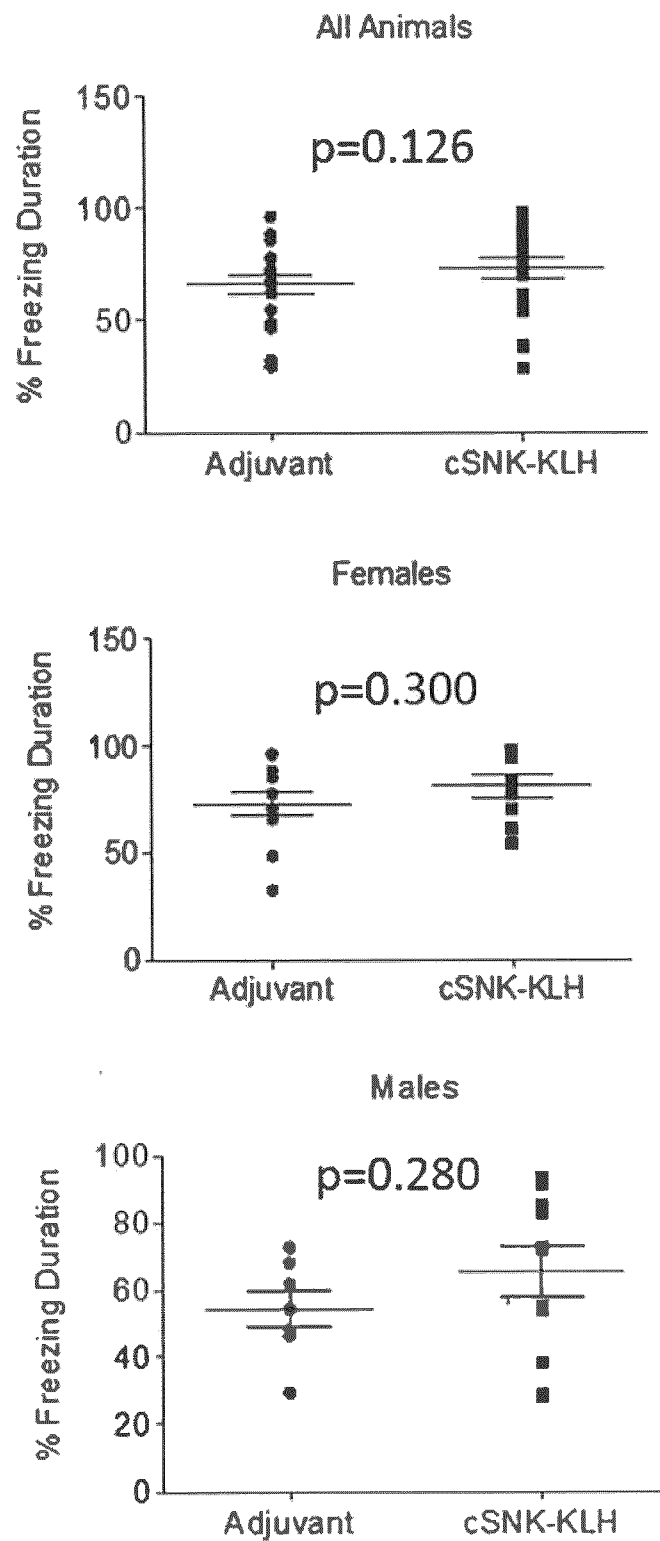


Figure 9

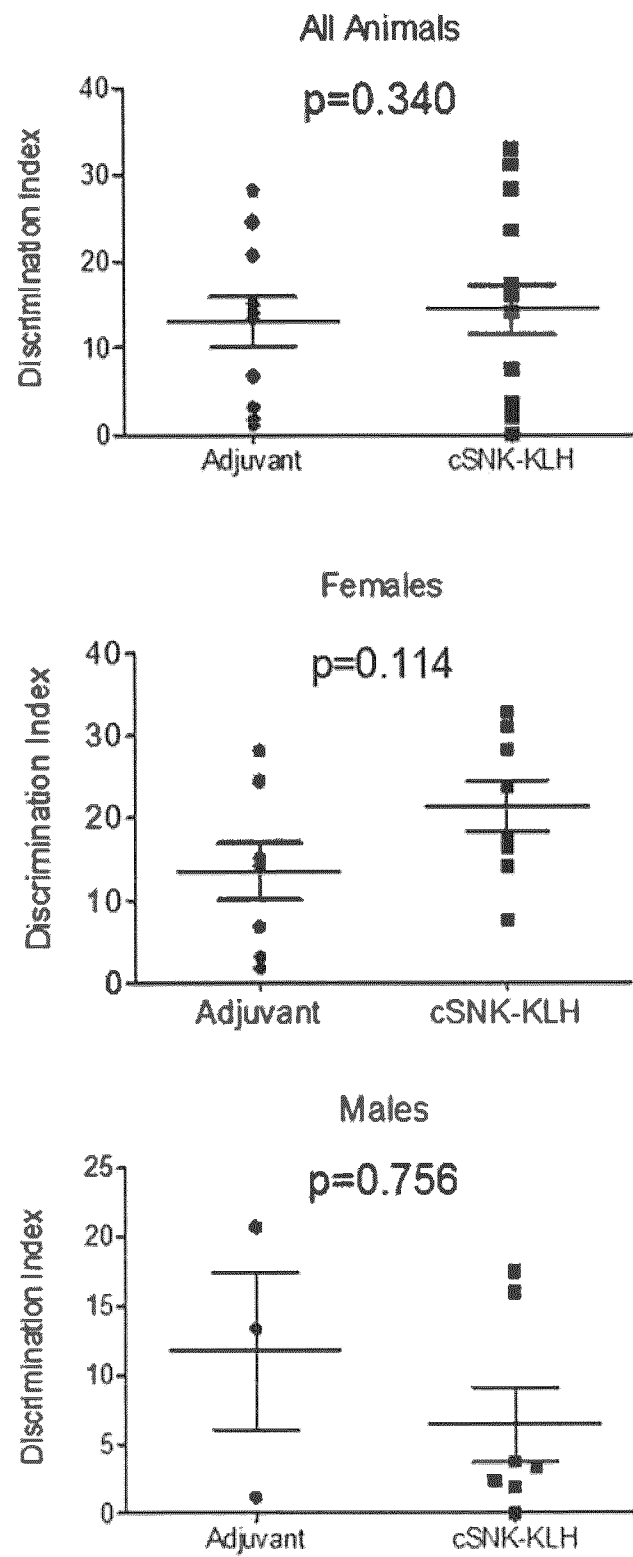


Figure 10

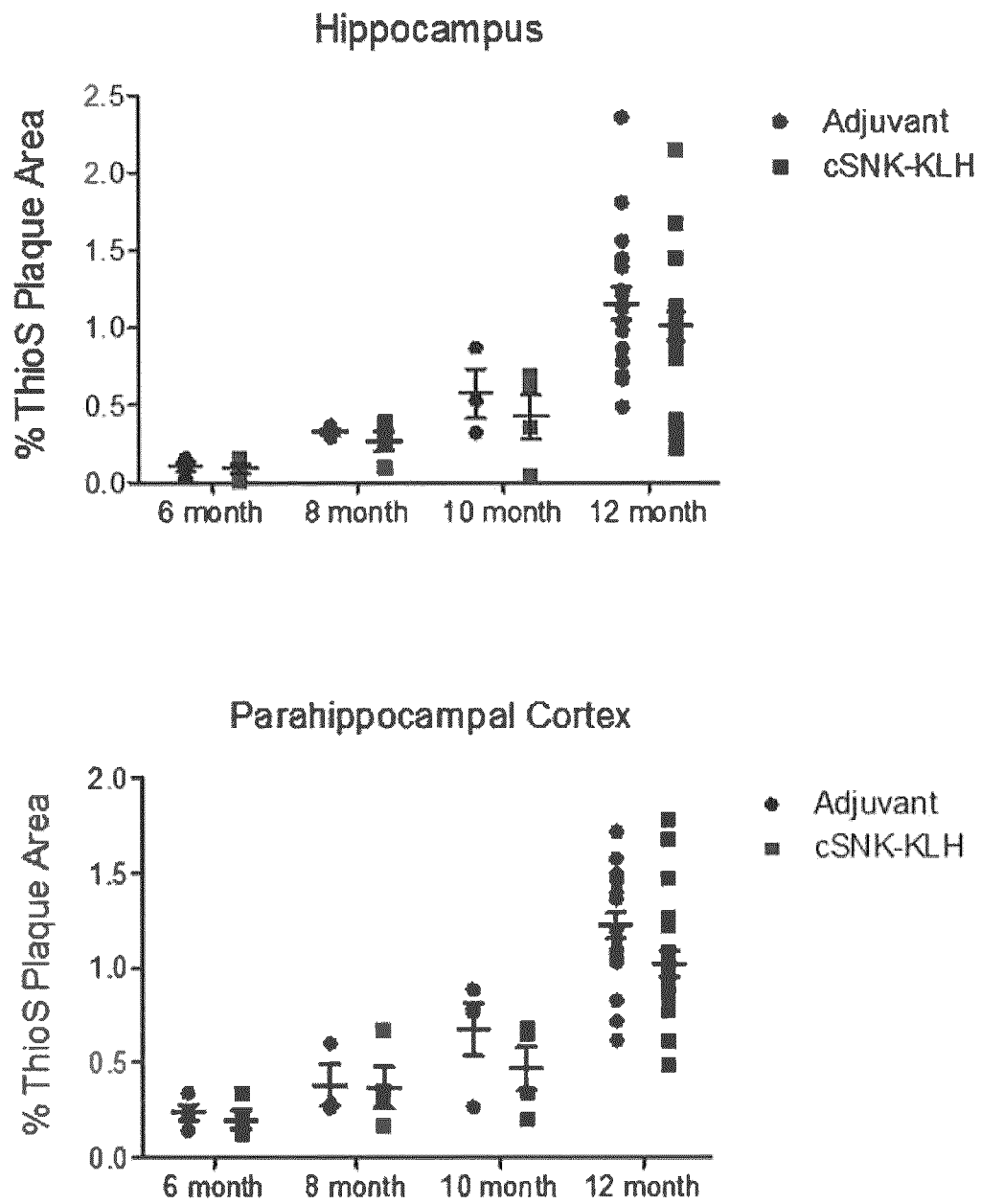


Figure 11

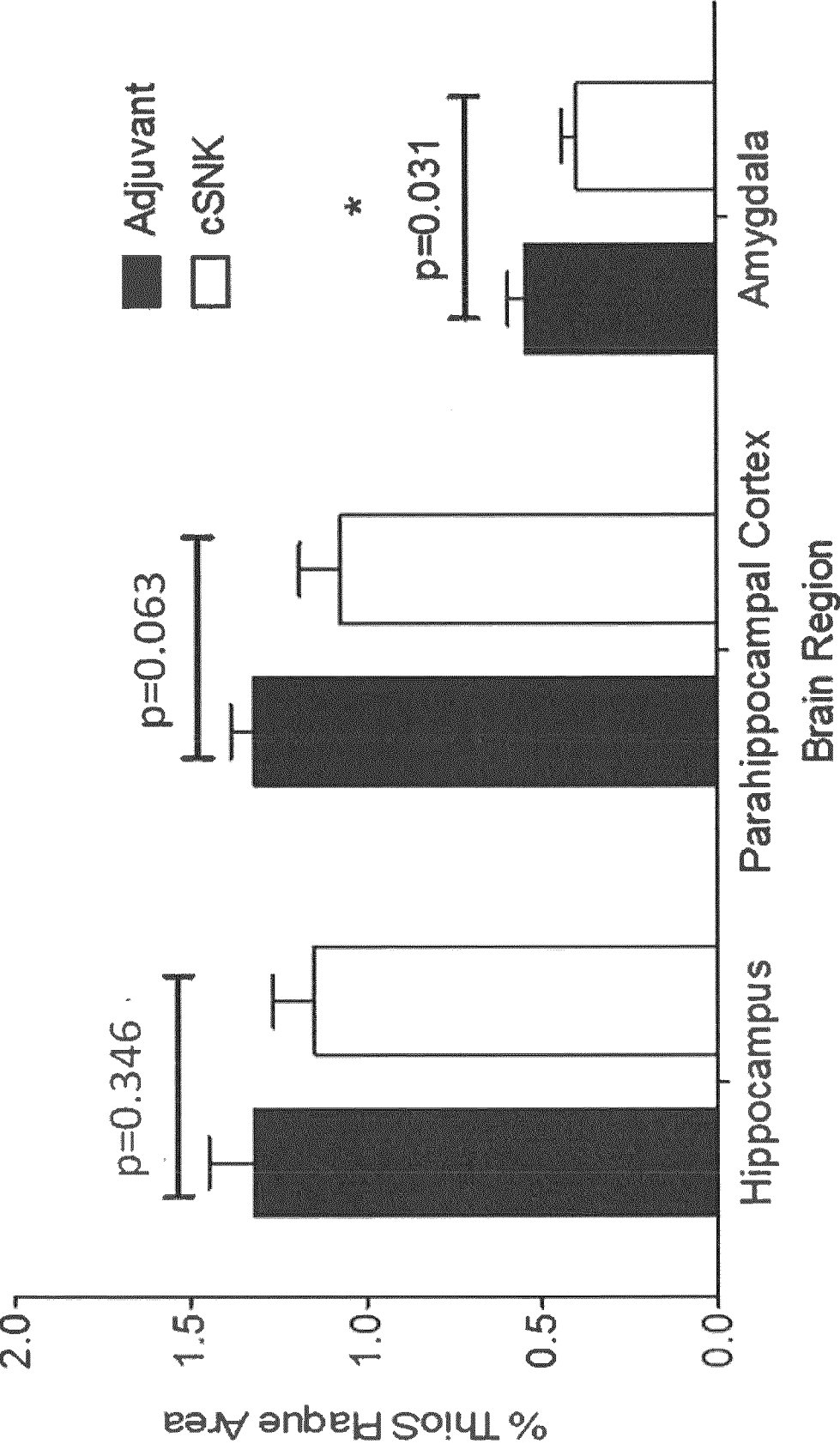


Figure 12

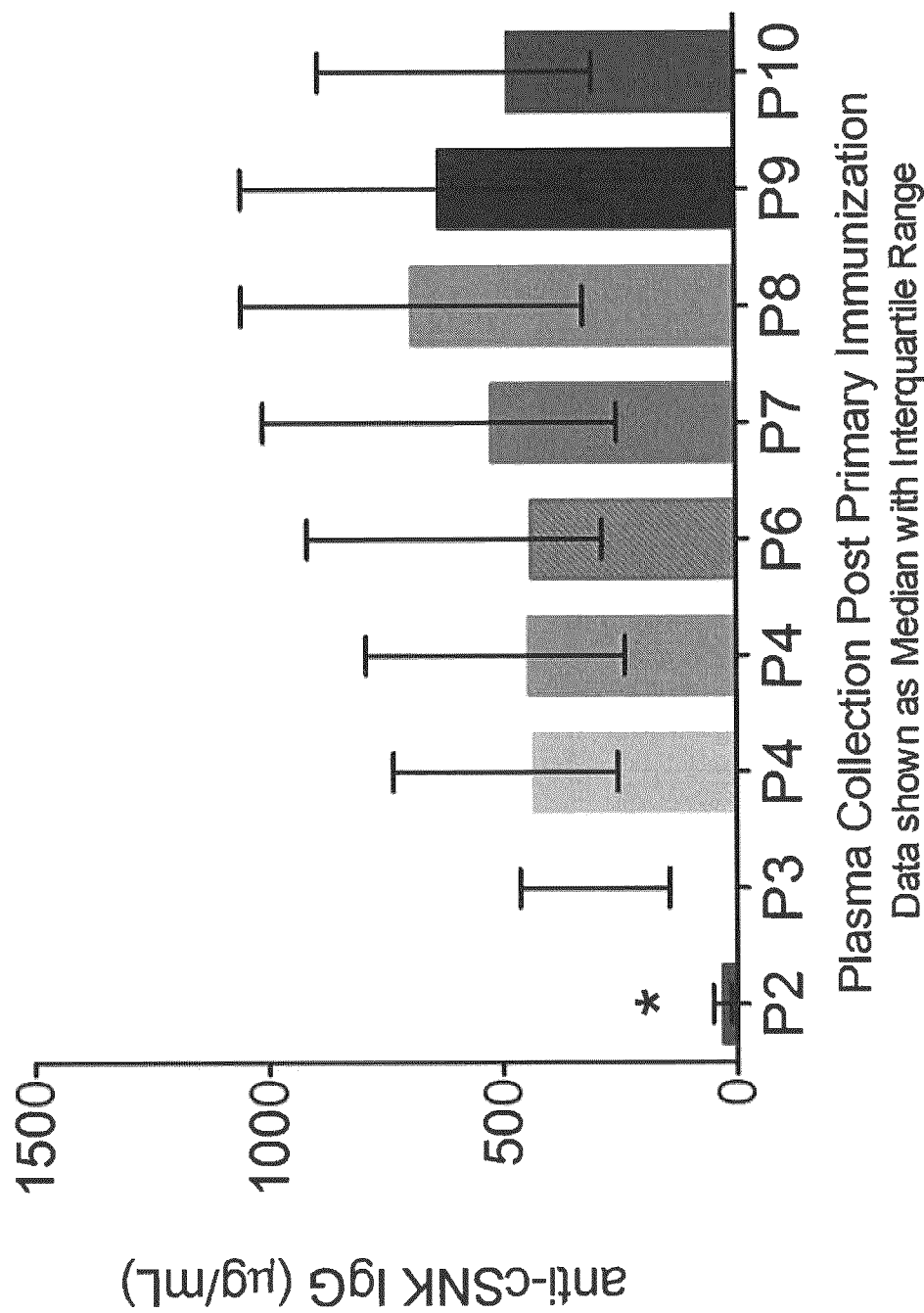


Figure 13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2015/050659

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **A61K 38/12** (2006.01), **A61K 39/00** (2006.01), **A61K 39/39** (2006.01), **A61K 47/48** (2006.01),
A61P 25/28 (2006.01), **C07K 19/00** (2006.01) (more IPCs on the last page)

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC: A61K 38/12 (2006.01), A61K 39/00 (2006.01), A61K 39/39 (2006.01), A61K 47/48 (2006.01),
A61P 25/28 (2006.01), C07K 19/00 (2006.01), C07K 7/64 (2006.01), C07K 14/47 (2006.01)

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

Electronic database(s) consulted during the international search (name of database(s) and, where practicable, search terms used)

Google and Google Scholar: 1) cyclic AND "CGSNKGG" 2) cyclic AND cashman AND "CGSNKGG" 3) cyclic AND Alzheimer AND nykiforuk **Pubmed:** 1) cyclic AND Alzheimer 2) Cashman AND cyclic AND Alzheimer **Questel Orbit:** 1) ((CYCLIC)/CLMS AND (PEPTIDE AND AMYLOID AND SNK AND ALZHEIMER)/DESC/ODES) **Scopus:** 1) cyclic AND Alzheimer

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO2013/071267A1 (CASHMAN, NR and MCLARTY, G) 16 May 2013 (16-05-2013), the whole document.	1-29
Y	WO2011/106885A1 (CASHMAN, NR) 9 September 2011 (09-09-2011), the whole document.	1-29
A	SILVERMAN, J. An immunological epitope specific for toxic oligomeric A β in Alzheimer's disease. <i>Alzheimers Dement.</i> July 2012, Vol. 8, issue 4, page P625, <DOI: 10.1016/j.jalz.2012.05.1666, the whole document	1-29
A	HOOGERHOUT, P. Cyclic Undecamer Peptide Mimics a Turn in Folded Alzheimer Amyloid b and Elicits Antibodies against Oligomeric and Fibrillar Amyloid and Plaques. <i>PLoS One.</i> 19 April 2011, Vol. 6, issue 4, e19110 [online], <DOI: 10.1371/journal.pone.0019110, eISSN 1932-6203, the whole document.	1-29

☐ Further documents are listed in the continuation of Box C.

☒ See patent family annex.

* Special categories of cited documents:	"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A" document defining the general state of the art which is not considered to be of particular relevance	"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E" earlier application or patent but published on or after the international filing date	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&" document member of the same patent family
"O" document referring to an oral disclosure, use, exhibition or other means	
"P" document published prior to the international filing date but later than the priority date claimed	

Date of the actual completion of the international search
10 September 2015 (10-09-2015)

Date of mailing of the international search report
22 September 2015 (22-09-2015)

Name and mailing address of the ISA/CA
Canadian Intellectual Property Office
Place du Portage I, C114 - 1st Floor, Box PCT
50 Victoria Street
Gatineau, Quebec K1A 0C9
Facsimile No.: 001-819-953-2476

Authorized officer

Marcel Brisebois (613) 697-5240

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA2015/050659**Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of the first sheet)**

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim Nos.: 1-23
because they relate to subject matter not required to be searched by this Authority, namely:
Claims 1-23 are directed to a method for treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search under Rule 39.1(iv) of the PCT. However, this Authority has carried out a search based on the alleged effect or purpose/use of the product(s) recited in claims 1-23.
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claim Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.

PCT/CA2015/050659

Patent Document Cited in Search Report	Publication Date	Patent Family Member(s)	Publication Date
WO2011106885A1	09 September 2011 (09-09-2011)	WO2011106885A1	09 September 2011 (09-09-2011)
		AU20111223456A1	18 October 2012 (18-10-2012)
		AU2012335014A1	19 June 2014 (19-06-2014)
		CA2791538A1	09 September 2011 (09-09-2011)
		CA2855669A1	16 May 2013 (16-05-2013)
		CL2012002438A1	13 September 2013 (13-09-2013)
		CN102858796A	02 January 2013 (02-01-2013)
		CN104093454A	08 October 2014 (08-10-2014)
		CO6640211A2	22 March 2013 (22-03-2013)
		EP2542571A1	09 January 2013 (09-01-2013)
		EP2542571A4	31 July 2013 (31-07-2013)
		EP2780082A1	24 September 2014 (24-09-2014)
		JP2013524774A	20 June 2013 (20-06-2013)
		KR20130045850A	06 May 2013 (06-05-2013)
		MX2012010175A	23 November 2012 (23-11-2012)
		NZ602548A	24 December 2014 (24-12-2014)
		RU2012139043A	10 April 2014 (10-04-2014)
		SG183854A1	30 October 2012 (30-10-2012)
		US2013084283A1	04 April 2013 (04-04-2013)
		US2014314773A1	23 October 2014 (23-10-2014)
		WO2013071267A1	16 May 2013 (16-05-2013)
WO2013071267A1	16 May 2013 (16-05-2013)	WO2013071267A1	16 May 2013 (16-05-2013)
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		SG183854A1	30 October 2012 (30-10-2012)
		US2013084283A1	04 April 2013 (04-04-2013)
		US2014314773A1	23 October 2014 (23-10-2014)
		WO2011106885A1	09 September 2011 (09-09-2011)

C07K 7/64 (2006.01), *C07K 14/47* (2006.01)