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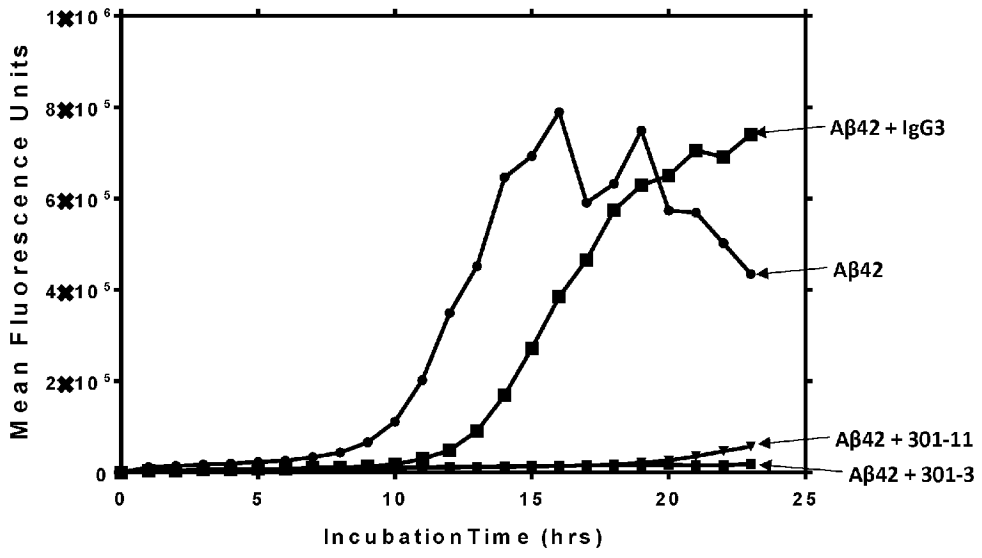
(54) Title: ANTIBODIES TO AMYLOID BETA

Figure 1



(57) Abstract: The disclosure pertains to antibodies that bind A-beta oligomers and methods of making and using said antibodies.

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[Continued on next page]

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Title: Antibodies to Amyloid beta

FAMILY DETAIL

This International PCT application claims the benefit of priority to U.S provisional application serial number 62/363566 filed July 18, 2016, International application PCT/CA2016/051303 filed November 9th, 2016, U.S provisional Serial no. 62/507587 filed May 17, 2017 and U.S provisional Serial no. 62/507633 filed May 17, 2017, each of which are herein incorporated by reference in their entirety.

Field

[0001] The present disclosure relates to antibodies that are selective for Amyloid beta (A-beta or Aβ) oligomers as well as compositions and uses thereof.

Background

[0002] Amyloid-beta (A-beta), which exists as a 36-43 amino acid peptide, is a product released from amyloid precursor protein (APP) by the enzymes β and γ secretase. In AD patients, A-beta can be present in soluble monomers, insoluble fibrils and soluble oligomers. In monomer form, A-beta exists as a predominantly unstructured polypeptide chain. In fibril form, A-beta can aggregate into distinct morphologies, often referred to as strains. Several of these structures have been determined by solid-state NMR.

[0003] For, example, structures for several strains of fibrils are available in the Protein Data Bank (PDB), a crystallographic database of atomic resolution three dimensional structural data, including a 3-fold symmetric A*β* structure (PDB entry, 2M4J); a two-fold symmetric structure of A*β*-40 monomers (PDB entry 2LMN), and a single-chain, parallel in-register structure of A*β*-42 monomers (PDB entry 2MXU).

[0004] The structure of 2M4J is reported in Lu et al [8], and the structure of 2MXU is reported in Xiao et al [9]. The structure of 2LMN is reported in Petkova et al [10].

[0005] A-beta oligomers have been shown to kill cell lines and neurons in culture and block a critical synaptic activity that subserves memory, referred to as long term potentiation (LTP), in slice cultures and living animals.

[0006] The structure of the oligomer has not been determined to date. Moreover, NMR and other evidence indicates that the oligomer exists not in a single well-defined structure, but in a conformationally-plastic, malleable structural ensemble with limited regularity. Moreover, the concentration of toxic oligomer species is far below either that of the monomer or fibril (estimates vary but are on the order of 1000-fold below or more), making this target elusive.

[0007] Antibodies that bind A-beta have been described.

[0008] WO2009048538A2 titled USE OF ANTI-AMYLOID ANTIBODY IN OCULAR DISEASES discloses chimeric antibodies that recognize one or more binding sites on A-beta and are useful for the treatment for ocular diseases.

[0009] US9221812B2 titled COMPOUNDS FOR THE TREATMENT OF DISEASES ASSOCIATED WITH AMYLOID OR AMYLOID-LIKE PROTEINS describes pharmaceutical

compositions and discontinuous antibodies that bind A-beta including an epitope between amino acid residues 12 to 24 for the treatment of amyloid-related diseases.

[0010] WO2003070760A2 titled ANTI-AMYLOID BETA ANTIBODIES AND THEIR USE discloses antibodies that recognize an A-beta discontinuous epitope, wherein the first region comprises the amino acid sequence AEFRHDSGY or a fragment thereof and wherein the second region comprises the amino acid sequence VHHQKLVFFAEDVG or a fragment thereof.

[0011] US20110171243A1 titled COMPOUNDS TREATING AMYLOIDOSES discloses a peptide mimotope capable of inducing the *in vivo* formation of antibodies that bind HQKLVFand/or HQKLVFFAED, and its use.

[0012] WO2008088983A1 and WO2001062801A2 disclose a pegylated antibody fragment that binds A-beta amino acids 13-28 and its use in treating A-beta related diseases. Solanezumab and Crenezumab bind amino acids 16-26 on A-beta. Crenezumab interacts with the monomer, oligomer and fibril. Midregion antibodies, including solanezumab (picomolar affinity) and crenezumab (nanomolar affinity), appear to preferentially bind monomeric A-beta [13].

[0013] WO2009149487A2 titled COMPOUNDS FOR TREATING SYMPTOMS ASSOCIATED WITH PARKINSON'S DISEASE describes compounds comprising a peptide having binding capacity for an antibody specific for an A-beta epitope such as EVHHQKL, HQKLVF and HQKLVFFAED.

[0014] The HHQK (SEQ ID NO: 7) domain is described as involved in plaque induction of neurotoxicity in human microglia, as described in Giulian D *et al.* [11] and Winkler *et al.* [12]. Non-antibody therapeutic agents that bind HHQK (SEQ ID NO: 7) have been disclosed for the treatment of protein folding diseases (US20150105344A1, WO2006125324A1).

[0015] U.S. patents 5,766,846; 5,837,672; and 5,593,846 (which are incorporated herein by reference) describe the production of murine monoclonal antibodies to the central domain of the Aβ peptide. WO 01/62801 describes antibodies that bind A-beta between amino acids 13-28. WO2004071408 discloses humanized antibodies.

[0016] WO2009086539A2 titled TREATMENT AND PROPHYLAXIS OF AMYLOIDOSIS is directed to Amyloidosis and amyloid light chain amyloidosis, by administering peptides comprising neoepitopes, such as amyloid protein A (AA) fragments from a C-terminal region of AA, and antibodies specific for neoepitopes of aggregated amyloid proteins, for example, antibodies specific for the C-terminal region of AA fibrils.

[0017] WO2003070760 titled ANTI-AMYLOID BETA ANTIBODIES AND THEIR USE is directed towards antibody molecules capable of specifically recognizing two regions of the R-A4 peptide, wherein the first region comprises the amino acid sequence AEFRHDSGY or a fragment thereof and wherein the second region comprises the amino acid sequence VHHAEDVFFAEDVG or a fragment thereof.

[0018] WO2006066089 titled HUMANIZED AMYLOID BETA ANTIBODIES FOR USE IN IMPROVING COGNITION is directed to improved agents and methods for treatment of diseases associated with beta amyloid and in particular to the identification and characterization of a monoclonal antibody, 12A11, that specifically binds to Aβ peptide and is effective at reducing plaque burden associated with amyloidogenic disorders (e.g., AD).

[0019] WO2007068429 titled ANTIBODIES AGAINST AMYLOID BETA 4 WITH GLYCOSYLATED IN THE VARIABLE REGION is directed to a purified antibody molecule preparation being characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V_H).

[0020] WO 03/016466 is directed variant 266 antibodies that are engineered to lack an N-glycosylation site within the CDR2 of the heavy chain, pharmaceutical compositions thereof, and polynucleotide sequences, vectors, and transformed cells useful to express the variant antibodies. The variants are described to sequester soluble A-beta peptide from human biological fluids and specifically bind an epitope contained within position 13-28 of the amyloid beta peptide.

[0021] Yu et al. describes a hexavalent foldable Aβ1-15 (6Aβ15) fused to PADRE or toxin-derived carrier proteins. Wang et al 2016 report that peripheral administration of this antibody mitigates Alzheimer's disease like pathology and cognitive decline in a transgenic animal of aged Alzheimer Disease [14], [15].

[0022] Antibodies that preferentially or selectively bind A-beta oligomers over monomers or over fibrils or over both monomers and fibrils are desirable.

Summary

[0023] An aspect includes an isolated antibody comprising a light chain variable region and a heavy chain variable region, optionally fused, the heavy chain variable region comprising complementarity determining regions CDR-H1, CDR-H2 and CDR-H3, the light chain variable region comprising complementarity determining region CDR-L1, CDR-L2 and CDR-L3 and with the amino acid sequences of said CDRs comprising or consisting of the sequences SEQ ID Nos: 1-6; or SEQ ID Nos:1,2, 80 and 4-6, or SEQ ID Nos: 1, 2, 80-83, for example as shown in Table 2.

[0024] In an embodiment, the isolated antibody is conformation specific and/or selective.

[0025] In an embodiment, an antibody described herein, optionally the antibody having CDRs comprising or consisting of SEQ ID Nos:1-6 or SEQ ID Nos:1,2, 80 and 4-6 or SEQ ID Nos: 1, 2, 80-83, selectively binds to a cyclic compound comprising HHQK (SEQ ID NO: 7) over a corresponding linear peptide, optionally wherein the antibody is at least 2 fold, 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 100 fold, at least 500 fold, at least 1000 fold more selective for the cyclic compound over the corresponding linear compound.

[0026] In another embodiment, an antibody described herein, optionally the antibody having CDRs comprising or consisting of SEQ ID Nos:1-6 or SEQ ID Nos:1,2, 80 and 4-6 or SEQ ID Nos: 1,

2, 80-83 selectively binds does not specifically and/or selectively bind a linear peptide comprising sequence HHQK (SEQ ID NO: 7), optionally wherein the sequence of the linear peptide is a linear version of a cyclic compound used to raise the antibody.

[0027] In another embodiment, the antibody having a CDR set as listed in Table 2, selectively binds A-beta oligomer over A-beta monomer and/or A-beta fibril.

[0028] In another embodiment, the selectivity is at least 2 fold, at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 100 fold, at least 500 fold, at least 1000 fold more selective for A-beta oligomer over A-beta monomer and/or A-beta fibril.

[0029] In another embodiment, the antibody lacks or has negligible binding to A-beta fibril plaques in situ.

[0030] In another embodiment, the antibody is a monoclonal antibody or a polyclonal antibody.

[0031] In another embodiment, the antibody having CDRs comprising or consisting of SEQ ID Nos:1-6 or SEQ ID Nos:1,2, 80 and 4-6 or or SEQ ID Nos: 1, 2, 80-83 is a humanized antibody. In an embodiment, the humanized antibody has a heavy chain variable region having a sequence and/or a light chain variable region having a sequence selected from the sequences in Table 4A.

[0032] Also provided in another aspect, is a hybridoma expressing an antibody comprising CDRs described in Table 2.

[0033] A further aspect is a humanized antibody wherein the humanized antibody has a heavy chain variable region having a sequence and/or a light chain variable region having a sequence selected from the sequences in Table 4B.

[0034] In an embodiment, the humanized antibody selectively or specifically binds a cyclic peptide having sequence of SEQ ID NO: 12, relative to a linear peptide of the same sequence or selectively or specifically binds oligomeric Abeta relative to A-beta monomer and/or A-beta fibril.

[0035] In another embodiment, the antibody is an antibody binding fragment of an antibody described herein selected from Fab, Fab', F(ab')2, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies, diabodies, and multimers thereof.

[0036] An aspect includes immunoconjugate comprising the antibody described herein and a detectable label or cytotoxic agent.

[0037] In an embodiment, the detectable label comprises a positron emitting radionuclide, optionally for use in subject imaging such as PET imaging.

[0038] An aspect includes a composition comprising the antibody described herein, or the immunoconjugate described herein, optionally with a diluent.

[0039] An aspect includes a nucleic acid molecule encoding a proteinaceous portion of the compound or immunogen described herein, the antibody described herein or proteinaceous immunoconjugates described herein.

[0040] An aspect includes a vector comprising the nucleic acid described herein.

[0041] An aspect includes a cell expressing an antibody described herein, optionally wherein the cell is a hybridoma comprising the vector described herein.

[0042] An aspect includes a kit comprising the antibody described herein, the immunoconjugate described herein, the composition described herein, the nucleic acid molecule described herein, the vector described herein or the cell described herein.

[0043] An aspect includes a method of determining if a biological sample comprises A-beta, the method comprising:

a. contacting the biological sample with an antibody described herein or the immunoconjugate described herein; and

b. detecting the presence of any antibody complex.

[0044] In an embodiment, the biological sample contains A-beta oligomer the method comprising:

a. contacting the sample with the antibody described herein or the immunoconjugate described herein that is specific and/or selective for A-beta oligomers under conditions permissive for forming an antibody: A-beta oligomer complex; and

b. detecting the presence of any complex;

wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.

[0045] In another embodiment, the amount of complex is measured.

[0046] In another embodiment, the sample comprises brain tissue or an extract thereof, whole blood, plasma, serum and/or CSF.

[0047] In another embodiment, the sample is a human sample.

[0048] In another embodiment, the sample is compared to a control, optionally a previous sample.

[0049] In another embodiment, the level of A-beta is detected by an analytical assay including but not limited to SPR, Kinexa, Mesoscale, ELISA, Singulex, Luminex and Simoa.

[0050] An aspect includes a method of measuring a level of A-beta in a subject, the method comprising administering to a subject at risk or suspected of having or having AD, an immunoconjugate comprising an antibody described herein wherein the antibody is conjugated to a detectable label; and detecting the label, optionally quantitatively detecting the label.

[0051] In an embodiment, the label is a positron emitting radionuclide.

[0052] An aspect includes a method of inhibiting A-beta oligomer propagation, the method comprising contacting a cell or tissue expressing A-beta with or administering to a subject in need thereof an effective amount of an A-beta oligomer specific or selective antibody or immunoconjugate described herein, to inhibit A-beta aggregation and/or oligomer propagation.

[0053] An aspect includes a method of treating AD and/or other A-beta amyloid related diseases, the method comprising administering to a subject in need thereof 1) an effective amount of an antibody or immunoconjugate described herein, or a pharmaceutical composition comprising said antibody; 2) a nucleic acid or vector comprising a nucleic acid encoding the antibody of 1, to a subject in need thereof.

[0054] In an embodiment, a biological sample from the subject to be treated is assessed for the presence or levels of A-beta using an antibody described herein.

[0055] In another embodiment, the antibody, immunoconjugate, composition or nucleic acid or vector is administered directly to the brain or other portion of the CNS.

[0056] Other features and advantages of the present disclosure will become apparent from the following detailed description. It should be understood, however, that the detailed description and the specific examples while indicating preferred embodiments of the disclosure are given by way of illustration only, since various changes and modifications within the spirit and scope of the disclosure will become apparent to those skilled in the art from this detailed description.

Brief description of the drawings

[0057] An embodiment of the present disclosure will now be described in relation to the drawings in which:

[0058] FIG. 1 Graph reporting effect of antibodies on propagation of A-beta oligomers in vitro.

Detailed description of the Disclosure

[0059] Provided herein are antibodies comprising CDRs having sequences as shown in Table 2, and/or having variable region sequences provided in any of Tables 3, 4A and 4B and/or the sequences in Table 8 are described, immunotherapeutic compositions thereof and methods of use thereof. Said antibodies may target epitopes preferentially accessible in toxic oligomeric species of A-beta, including oligomeric species associated with Alzheimer's disease (AD).

[0060] As shown in the Examples, antibodies raised using a cyclic peptide comprising HHQK (SEQ ID NO: 7), preferentially bound oligomeric Abeta and/or selectively bound the cyclic peptide compared to a linear peptide of the same sequence (e.g. corresponding linear sequence).

Experimental results are described and identify epitope-specific and conformationally selective antibodies that bind synthetic oligomer selectively compared to synthetic monomers, bind CSF from AD patients preferentially over control CSF and/or bind soluble brain extract from AD patients preferentially over control soluble brain extract. Further staining of AD brain tissue identified antibodies that show no or negligible plaque binding and in vitro studies found that the antibodies inhibited Aβ oligomer propagation and aggregation.

I. Definitions

[0061] As used herein, the term 'A-beta' may alternately be referred to as 'amyloid beta', 'amyloid β', A-beta, A-beta or 'Aβ'. Amyloid beta is a peptide of 36-43 amino acids and includes all wild-type and mutant forms of all species, particularly human A-beta. A-beta40 refers to the 40 amino acid form; A-beta42 refers to the 42 amino acid form, etc. The amino acid sequence of human wildtype A-beta42 is shown in SEQ ID NO: 73.

[0062] As used herein, the term "A-beta monomer" herein refers to any of the individual subunit forms of the A-beta (e.g. 1-40, 1-42, 1-43) peptide.

[0063] As used herein, the term "A-beta oligomer" herein refers to a plurality of any of the A-beta subunits wherein several (e.g. at least two) A-beta monomers are non-covalently aggregated in a conformationally-flexible, partially-ordered, three-dimensional globule of less than about 100, or more typically less than about 50 monomers. For example, an oligomer may contain 3 or 4 or 5 or more monomers. The term "A-beta oligomer" as used herein includes both synthetic A-beta oligomer and/or native A-beta oligomer. "Native A-beta oligomer" refers to A-beta oligomer formed in vivo, for example in the brain and CSF of a subject with AD.

[0064] As used herein, the term "A-beta fibril" refers to a molecular structure that comprises assemblies of non-covalently associated, individual A-beta peptides which show fibrillary structure under an electron microscope. The fibrillary structure is typically a "cross beta" structure; there is no theoretical upper limit on the size of multimers, and fibrils may comprise thousands or many thousands of monomers. Fibrils can aggregate by the thousands to form senile plaques, one of the primary pathological morphologies diagnostic of AD.

[0065] The term "HHQK" means the amino acid sequence histidine, histidine, glutamine, lysine, as shown in SEQ ID NO: 7. Depending on the context, the reference of the amino acid sequence can refer to a sequence in A-beta or an isolated peptide, such as the amino acid sequence of a cyclic compound.

[0066] The term "amino acid" includes all of the naturally occurring amino acids as well as modified L-amino acids. The atoms of the amino acid can include different isotopes. For example, the amino acids can comprise deuterium substituted for hydrogen nitrogen-15 substituted for nitrogen-14, and carbon-13 substituted for carbon-12 and other similar changes.

[0067] The term "antibody" as used herein is intended to include, monoclonal antibodies, polyclonal antibodies, single chain, veneered, humanized and other chimeric antibodies and binding

fragments thereof, including for example a single chain Fab fragment, Fab'2 fragment or single chain Fv fragment. The antibody may be from recombinant sources and/or produced in animals such as rabbits, llamas, sharks etc. Also included are human antibodies that can be produced in transgenic animals or using biochemical techniques or can be isolated from a library such as a phage library. Humanized or other chimeric antibodies may include sequences from one or more than one isotype or class or species.

[0068] The phrase "isolated antibody" refers to antibody produced in vivo or in vitro that has been removed from the source that produced the antibody, for example, an animal, hybridoma or other cell line (such as recombinant insect, yeast or bacteria cells that produce antibody). The isolated antibody is optionally "purified", which means at least: 80%, 85%, 90%, 95%, 98% or 99% purity.

[0069] The term "binding fragment" as used herein to a part or portion of an antibody or antibody chain comprising fewer amino acid residues than an intact or complete antibody or antibody chain and which binds the antigen or competes with intact antibody. Exemplary binding fragments include without limitations Fab, Fab', F(ab')2, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies, diabodies, and multimers thereof. Fragments can be obtained via chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. For example, F(ab')2 fragments can be generated by treating the antibody with pepsin. The resulting F(ab')2 fragment can be treated to reduce disulfide bridges to produce Fab' fragments. Papain digestion can lead to the formation of Fab fragments. Fab, Fab' and F(ab')2, scFv, dsFv, ds-scFv, dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be constructed by recombinant expression techniques.

[0070] The terms ”IMGT numbering” or “ImMunoGeneTics database numbering”, which are recognized in the art, refer to a system of numbering amino acid residues which are more variable (i.e. hypervariable) than other amino acid residues in the heavy and light chain variable regions of an antibody, or antigen binding portion thereof.

[0071] As used herein, the term "conformational epitope" refers to an epitope where the epitope amino acid sequence has a particular three-dimensional structure wherein at least an aspect of the three-dimensional structure not present or less likely to be present in another form for example a corresponding linear peptide or Abeta monomer and is specifically and/or selectively recognized by the cognate antibody. Antibodies which specifically bind a conformation-specific epitope recognize the spatial arrangement of one or more of the amino acids of that conformation-specific epitope. For example, an HHQK (SEQ ID NO: 7) conformational epitope refers to an epitope of HHQK (SEQ ID NO:7) that is recognized by antibodies selectively, for example at least 2 fold, 3 fold, 5 fold, 10 fold, 50 fold, 100 fold, 250 fold, 500 fold or 1000 fold or greater more selectivity as compared to antibodies raised using linear HHQK (SEQ ID NO: 7). When an antibody is said to selectively bind an epitope such as a conformational epitope, such as HHQK (SEQ ID NO: 7), what is meant is that the antibody preferentially binds one or more particular conformations containing the specified residues or a part thereof with greater affinity than it binds said residues in another conformation. For example, when an

antibody is said to selectively bind a cyclopeptide comprising HHQK or related epitope relative to a corresponding linear peptide, the antibody binds the cyclopeptide with at least a 2 fold greater affinity than it binds the linear peptide. Similarly, when an antibody is said to selectively bind oligomeric Abeta, the antibody binds the oligomeric species with at least a 2 fold greater affinity than it binds Abeta monomer and/or plaque fibrils.

[0072] The term “no or negligible plaque binding” or “lacks or has negligible plaque binding” as used herein with respect to an antibody means that the antibody does not show typical plaque morphology staining on immunohistochemistry (e.g. in situ, optionally as compared to plaque staining seen with Abeta antibody 6E10) and the level of staining is comparable to or no more than 2 fold the level seen with an IgG negative (e.g. irrelevant) isotype control.

[0073] The term "Isolated peptide" refers to peptide that has been produced, for example, by recombinant or synthetic techniques, and removed from the source that produced the peptide, such as recombinant cells or residual peptide synthesis reactants. The isolated peptide is optionally "purified", which means at least: 80%, 85%, 90%, 95%, 98% or 99% purity and optionally pharmaceutical grade purity.

[0074] The term “detectable label” as used herein refers to moieties such as peptide sequences (such a myc tag, HA-tag, V5-tag or NE-tag), fluorescent proteins that can be appended or introduced into a peptide or compound described herein and which is capable of producing, either directly or indirectly, a detectable signal. For example, the label may be radio-opaque, positron-emitting radionuclide (for example for use in PET imaging), or a radioisotope, such as ^{3}H, ^{13}N, ^{14}C, ^{18}F, ^{32}P, ^{35}S, ^{123}I, ^{125}I, ^{131}I; a fluorescent (fluorophore) or chemiluminescent (chromophore) compound, such as fluorescein isothiocyanate, rhodamine or luciferin; an enzyme, such as alkaline phosphatase, beta-galactosidase or horseradish peroxidase; an imaging agent; or a metal ion. The detectable label may be also detectable indirectly for example using secondary antibody.

[0075] The term “epitope” as commonly used means an antibody binding site, typically a polypeptide segment, in an antigen that is specifically recognized by the antibody. As used herein “epitope” can also refer to the amino acid sequences or part thereof identified on A-beta using the collective coordinates method described. For example an antibody generated against an isolated peptide corresponding to a cyclic compound comprising the identified target region HHQK SEQ ID NO:7), recognizes part or all of said epitope sequence. An epitope is "accessible” in the context of the present specification when it is accessible to binding by an antibody.

[0076] The term “greater affinity” as used herein refers to a relative degree of antibody binding where an antibody X binds to target Y more strongly (K_{on}) and/or with a smaller dissociation constant (K_{off}) than to target Z, and in this context antibody X has a greater affinity for target Y than for Z. Likewise, the term "lesser affinity" herein refers to a degree of antibody binding where an antibody X binds to target Y less strongly and/or with a larger dissociation constant than to target Z, and in this context antibody X has a lesser affinity for target Y than for Z. The affinity of binding between an antibody and its target antigen, can be expressed as K_A equal to $1/K_D$ where K_D is equal to k_{on}/k_{off}.

The k_{on} and k_{off} values can be measured using surface plasmon resonance technology, for example using a Molecular Affinity Screening System (MASS-1) (Sierra Sensors GmbH, Hamburg, Germany). An antibody that is selective for a conformation presented in a cyclic compound optional a cyclic peptide for example has a greater affinity for the cyclic compound (e.g. cyclic peptide) compared to a corresponding sequence in linear form (e.g. the sequence non-cyclized).

[0077] The term “corresponding linear compound” with regard to a cyclic compound refers to a compound, optionally a peptide, comprising or consisting of the same sequence or chemical moieties as the cyclic compound but in linear (i.e. non-cyclized) form, for example having properties as would be present in solution of a linear peptide. For example, the corresponding linear compound can be the synthesized peptide that is not cyclized.

[0078] As used herein "specifically binds" in reference to an antibody means that the antibody recognizes an epitope sequence and binds to its target antigen with a minimum affinity. For example a multivalent antibody binds its target with a K_D of at least 1e-6, at least 1e-7, at least 1e-8, at least 1e-9, or at least 1e-10. Affinities greater than at least 1e-8 may be preferred. For example the K_D may be in the nanomolar range or the picomolar range. An antigen binding fragment such as Fab fragment comprising one variable domain, may bind its target with a 10 fold or 100 fold less affinity than a multivalent interaction with a non-fragmented antibody.

[0079] The term “selectively binds” as used herein with respect to an antibody that selectively binds a form of A-beta (e.g. fibril, monomer or oligomer) or a cyclic compound means that the antibody binds the form with at least 2 fold, at least 3 fold, or at least 5 fold, at least 10 fold, at least 100 fold, at least 250 fold, at least 500 fold or at least 1000 fold or more greater affinity. Accordingly an antibody that is more selective for a particular conformation (e.g. oligomer) preferentially binds the particular form of A-beta with at least 2 fold etc. greater affinity compared to another form and/or a linear peptide.

[0080] The term “animal” or "subject" as used herein includes all members of the animal kingdom including mammals, optionally including or excluding humans.

[0081] A "conservative amino acid substitution" as used herein, is one in which one amino acid residue is replaced with another amino acid residue without abolishing the protein's desired properties. Suitable conservative amino acid substitutions can be made by substituting amino acids with similar hydrophobicity, polarity, and R-chain length for one another. Examples of conservative amino acid substitution include:

Conservative Substitutions	
Type of Amino Acid	Substitutable Amino Acids
Hydrophilic	Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr
Sulphydryl	Cys
Aliphatic	Val, Ile, Leu, Met
Basic	Lys, Arg, His

Conservative Substitutions	
Aromatic	Phe, Tyr, Trp

[0082] The term "sequence identity" as used herein refers to the percentage of sequence identity between two polypeptide sequences or two nucleic acid sequences. To determine the percent identity of two amino acid sequences or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in the sequence of a first amino acid or nucleic acid sequence for optimal alignment with a second amino acid or nucleic acid sequence). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences (i.e., % identity=number of identical overlapping positions/total number of positions.times.100%). In one embodiment, the two sequences are the same length. The determination of percent identity between two sequences can also be accomplished using a mathematical algorithm. A preferred, non-limiting example of a mathematical algorithm utilized for the comparison of two sequences is the algorithm of Karlin and Altschul, 1990, Proc. Natl. Acad. Sci. U.S.A. 87:2264-2268, modified as in Karlin and Altschul, 1993, Proc. Natl. Acad. Sci. U.S.A. 90:5873-5877. Such an algorithm is incorporated into the NBLAST and XBLAST programs of Altschul et al., 1990, J. Mol. Biol. 215:403. BLAST nucleotide searches can be performed with the NBLAST nucleotide program parameters set, e.g., for score=100, word length=12 to obtain nucleotide sequences homologous to a nucleic acid molecules of the present application. BLAST protein searches can be performed with the XBLAST program parameters set, e.g., to score-50, word length=3 to obtain amino acid sequences homologous to a protein molecule described herein. To obtain gapped alignments for comparison purposes, Gapped BLAST can be utilized as described in Altschul et al., 1997, Nucleic Acids Res. 25:3389-3402. Alternatively, PSI-BLAST can be used to perform an iterated search which detects distant relationships between molecules (Id.). When utilizing BLAST, Gapped BLAST, and PSI-Blast programs, the default parameters of the respective programs (e.g., of XBLAST and NBLAST) can be used (see, e.g., the NCBI website). Another preferred non-limiting example of a mathematical algorithm utilized for the comparison of sequences is the algorithm of Myers and Miller, 1988, CABIOS 4:11-17. Such an algorithm is incorporated in the ALIGN program (version 2.0) which is part of the GCG sequence alignment software package. When utilizing the ALIGN program for comparing amino acid sequences, a PAM120 weight residue table, a gap length penalty of 12, and a gap penalty of 4 can be used. The percent identity between two sequences can be determined using techniques similar to those described above, with or without allowing gaps. In calculating percent identity, typically only exact matches are counted.

[0083] For antibodies, percentage sequence identities can be determined when antibody sequences maximally aligned by IMGT or other (e.g. Kabat numbering convention). After alignment, if

a subject antibody region (e.g., the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

[0084] The term “nucleic acid sequence” as used herein refers to a sequence of nucleoside or nucleotide monomers consisting of naturally occurring bases, sugars and intersugar (backbone) linkages. The term also includes modified or substituted sequences comprising non-naturally occurring monomers or portions thereof. The nucleic acid sequences of the present application may be deoxyribonucleic acid sequences (DNA) or ribonucleic acid sequences (RNA) and may include naturally occurring bases including adenine, guanine, cytosine, thymidine and uracil. The sequences may also contain modified bases. Examples of such modified bases include aza and deaza adenine, guanine, cytosine, thymidine and uracil; and xanthine and hypoxanthine. The nucleic acid can be either double stranded or single stranded, and represents the sense or antisense strand. Further, the term "nucleic acid" includes the complementary nucleic acid sequences as well as codon optimized or synonymous codon equivalents. The term "isolated nucleic acid sequences" as used herein refers to a nucleic acid substantially free of cellular material or culture medium when produced by recombinant DNA techniques, or chemical precursors, or other chemicals when chemically synthesized. An isolated nucleic acid is also substantially free of sequences which naturally flank the nucleic acid (i.e. sequences located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived.

[0085] “Operatively linked” is intended to mean that the nucleic acid is linked to regulatory sequences in a manner which allows expression of the nucleic acid. Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes. Selection of appropriate regulatory sequences is dependent on the host cell chosen and may be readily accomplished by one of ordinary skill in the art. Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector.

[0086] The term "vector" as used herein comprises any intermediary vehicle for a nucleic acid molecule which enables said nucleic acid molecule, for example, to be introduced into prokaryotic and/or eukaryotic cells and/or integrated into a genome, and include plasmids, phagemids, bacteriophages or viral vectors such as retroviral based vectors, Adeno Associated viral vectors and the like. The term "plasmid" as used herein generally refers to a construct of extrachromosomal genetic material, usually a circular DNA duplex, which can replicate independently of chromosomal DNA.

[0087] By “at least moderately stringent hybridization conditions” it is meant that conditions are selected which promote selective hybridization between two complementary nucleic acid molecules in solution. Hybridization may occur to all or a portion of a nucleic acid sequence molecule. The hybridizing portion is typically at least 15 (e.g. 20, 25, 30, 40 or 50) nucleotides in length. Those skilled in the art will recognize that the stability of a nucleic acid duplex, or hybrids, is determined by the Tm, which in sodium containing buffers is a function of the sodium ion concentration and temperature (Tm = 81.5°C – 16.6 (Log10 [Na+]) + 0.41(%(G+C) – 600/l), or similar equation). Accordingly, the parameters in the wash conditions that determine hybrid stability are sodium ion concentration and temperature. In order to identify molecules that are similar, but not identical, to a known nucleic acid molecule a 1% mismatch may be assumed to result in about a 1°C decrease in Tm, for example if nucleic acid molecules are sought that have a >95% identity, the final wash temperature will be reduced by about 5°C. Based on these considerations those skilled in the art will be able to readily select appropriate hybridization conditions. In preferred embodiments, stringent hybridization conditions are selected. By way of example the following conditions may be employed to achieve stringent hybridization: hybridization at 5x sodium chloride/sodium citrate (SSC)/5x Denhardt’s solution/1.0% SDS at Tm - 5°C based on the above equation, followed by a wash of 0.2x SSC/0.1% SDS at 60°C. Moderately stringent hybridization conditions include a washing step in 3x SSC at 42°C. It is understood, however, that equivalent stringencies may be achieved using alternative buffers, salts and temperatures. Additional guidance regarding hybridization conditions may be found in: Current Protocols in Molecular Biology, John Wiley & Sons, N.Y., 2002, and in: Sambrook et al., Molecular Cloning: a Laboratory Manual, Cold Spring Harbor Laboratory Press, 2001.

[0088] The term "treating" or “treatment” as used herein and as is well understood in the art, means an approach for obtaining beneficial or desired results, including clinical results. Beneficial or desired clinical results can include, but are not limited to, alleviation or amelioration of one or more symptoms or conditions, diminishment of extent of disease, stabilized (i.e. not worsening) state of disease, preventing spread of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, diminishment of the reoccurrence of disease, and remission (whether partial or total), whether detectable or undetectable. "Treating" and "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. "Treating" and "treatment" as used herein also include prophylactic treatment. For example, a subject with early stage AD can be treated to prevent progression can be treated with a compound, antibody, immunogen, nucleic acid or composition described herein to prevent progression.

[0089] The term "administered" as used herein means administration of a therapeutically effective dose of a compound or composition of the disclosure to a cell or subject.

[0090] As used herein, the phrase "effective amount" means an amount effective, at dosages and for periods of time necessary to achieve a desired result. Effective amounts when

administered to a subject may vary according to factors such as the disease state, age, sex, weight of the subject. Dosage regime may be adjusted to provide the optimum therapeutic response.

[0091] The term "pharmaceutically acceptable" means that the carrier, diluent, or excipient is compatible with the other components of the formulation and not substantially deleterious to the recipient thereof.

[0092] Compositions or methods "comprising" or "including" one or more recited elements may include other elements not specifically recited. For example, a composition that "comprises" or "includes" an antibody may contain the antibody alone or in combination with other ingredients.

[0093] In understanding the scope of the present disclosure, the term “consisting” and its derivatives, as used herein, are intended to be close ended terms that specify the presence of stated features, elements, components, groups, integers, and/or steps, and also exclude the presence of other unstated features, elements, components, groups, integers and/or steps.

[0094] The recitation of numerical ranges by endpoints herein includes all numbers and fractions subsumed within that range (e.g. 1 to 5 includes 1, 1.5, 2, 2.75, 3, 3.90, 4, and 5). It is also to be understood that all numbers and fractions thereof are presumed to be modified by the term "about." Further, it is to be understood that "a," "an," and "the" include plural referents unless the content clearly dictates otherwise. The term “about” means plus or minus 0.1 to 50%, 5-50%, or 10-40%, preferably 10-20%, more preferably 10% or 15%, of the number to which reference is being made.

[0095] Further, the definitions and embodiments described in particular sections are intended to be applicable to other embodiments herein described for which they are suitable as would be understood by a person skilled in the art. For example, in the following passages, different aspects of the invention are defined in more detail. Each aspect so defined may be combined with any other aspect or aspects unless clearly indicated to the contrary. In particular, any feature indicated as being preferred or advantageous may be combined with any other feature or features indicated as being preferred or advantageous.

[0096] The singular forms of the articles "a," "an," and "the" include plural references unless the context clearly dictates otherwise. For example, the term "a compound" or "at least one compound" can include a plurality of compounds, including mixtures thereof.

II. Antibodies and Nucleic acids

[0097] Disclosed herein are particular antibodies and uses thereof.

[0098] As demonstrated in the Examples, antibodies raised using cyclo(CGHHQKG) (SEQ ID NO: 12) were sequenced, selectively bound the cyclic compound relative to the corresponding linear peptide, selectively bound A-beta oligomer over monomer, and/or lacked appreciable plaque staining in AD tissue. Further said antibody was able to inhibit in vitro propagation of A-beta aggregation.

[0099] Accordingly an aspect includes an antibody comprising a light chain variable region and a heavy chain variable region, optionally fused, the heavy chain variable region comprising complementarity determining regions CDR-H1, CDR-H2 and CDR-H3, the light chain variable region comprising complementarity determining region CDR-L1, CDR-L2 and CDR-L3 and with the amino acid sequences of said CDRs comprising the sequences:

CDR-H1	GFTFSDYY	(SEQ ID NO: 1)
CDR-H2	ISDGGSYT	(SEQ ID NO: 2)
CDR-H3	ARDYYGSSSYTSGFAY	(SEQ ID NO: 3)
CDR-L1	QSLLNSRTRKNY	(SEQ ID NO: 4)
CDR-L2	WAS	(SEQ ID NO: 5)
CDR-L3	KQSYNLYT	(SEQ ID NO: 6)

[00100] In an embodiment, the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 9; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 9, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3.

[00101] In another embodiment, the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 11, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 11, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

[00102] In another embodiment, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in SEQ ID NO: 8 or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in SEQ ID NO: 10 or a codon degenerate or optimized version thereof.

[00103] In another embodiment, the heavy chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 9 and/or the light chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 11.

[00104] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or to human A-beta oligomers with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1-6.

[00105] In another embodiment, the antibody a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the heavy chain variable chain sequence of SEQ ID NO: 9 and/or the light chain variable region sequence of SEQ ID NO: 11.

[00106] Another aspect includes an isolated conformation specific and/or selective antibody comprising a light chain variable region and a heavy chain variable region, optionally fused, the heavy chain variable region comprising complementarity determining regions CDR-H1, CDR-H2 and CDR-H3, the light chain variable region comprising complementarity determining region CDR-L1, CDR-L2 and CDR-L3 and with the amino acid sequences of said CDRs comprising the sequences:

CDR-H1	GFTFSDYY	(SEQ ID NO: 1)
CDR-H2	ISDGGSYT	(SEQ ID NO: 2)
CDR-H3	ARDYYGSNSYTSGFAY	(SEQ ID NO: 80)
CDR-L1	QSLLNSRTRKNY	(SEQ ID NO: 4)
CDR-L2	WAS	(SEQ ID NO: 5)
CDR-L3	KQSYNLYT	(SEQ ID NO: 6).

[00107] In an embodiment, the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 85; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 85, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80.

[00108] In another embodiment, the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 87, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 89, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

[00109] In another embodiment, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in SEQ ID NO: 84 or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in SEQ ID NO: 86 or a codon degenerate or optimized version thereof.

[00110] In another embodiment, the heavy chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 85 and/or the light chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 87.

[00111] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1, 2, 80, 4-6.

[00112] In another embodiment, the antibody is an antibody that binds a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the heavy chain variable chain sequence of SEQ ID NO: 85 and/or the light chain variable region sequence of SEQ ID NO: 87.

[00113] Another aspect includes an isolated conformation specific and/or selective antibody comprising a light chain variable region and a heavy chain variable region, optionally fused, the heavy chain variable region comprising complementarity determining regions CDR-H1, CDR-H2 and CDR-H3, the light chain variable region comprising complementarity determining region CDR-L1, CDR-L2 and CDR-L3 and with the amino acid sequences of said CDRs comprising the sequences:

CDR-H1	GFTFSDYY	(SEQ ID NO: 1)
CDR-H2	ISDGGSYT	(SEQ ID NO: 2)
CDR-H3	ARDYYGSNSYTSGFAY	(SEQ ID NO: 80)
CDR-L1	QSIVHSNGNTY	(SEQ ID NO: 81)
CDR-L2	KVS	(SEQ ID NO: 82)
CDR-L3	FQGSHVPLT	(SEQ ID NO: 83).

[00114] In an embodiment, the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 85; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 85, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80.

[00115] In another embodiment, the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 89, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 89, wherein the CDR sequences are as set forth in SEQ ID NO: 81, 82 and 83, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 81, 82 and 83.

[00116] In another embodiment, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in SEQ ID NO: 84 or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in SEQ ID NO: 88 or a codon degenerate or optimized version thereof.

[00117] In another embodiment, the heavy chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 85 and/or the light chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID NO: 89.

[00118] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1, 2, 80-3.

[00119] In another embodiment, the antibody is an antibody that binds a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the heavy chain variable chain sequence of SEQ ID NO: 85 and/or the light chain variable region sequence of SEQ ID NO: 89.

[00120] In an embodiment, the antibody lacks binding a linear peptide comprising the sequence HHQK (SEQ ID NO: 7), optionally wherein the sequence of the linear peptide is a linear version of a cyclic sequence used to raise the antibody, optionally under conditions described in the Examples.

[00121] In an embodiment, the antibody specifically binds an epitope on A-beta as present in vivo, the epitope comprising or consisting HHQK (SEQ ID NO: 7), or a part thereof.

[00122] In an embodiment, the antibody does not specifically bind and/or is not selective for linear peptides consisting of HHQK (SEQ ID NO: 7). Selective binding can be measured using an ELISA or surface plasmon resonance measurement, as described herein.

[00123] In an embodiment, the antibody selectively binds a cyclic compound comprising HHQK (SEQ ID NO: 7) or a part thereof, optionally in the context of cyclo(CGHHQKG) (SEQ ID NO: 12) relative to a linear peptide comprising HHQK (SEQ ID NO: 7), optionally in the context of linear CGHHQKG (SEQ ID NO: 12). For example, in an embodiment the antibody selectively binds HHQK (SEQ ID NO: 7) in a cyclic conformation and has at least 2 fold, at least 5 fold, at least 10 fold at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 100 fold, at least 500 fold, at least 1000 fold more selective for HHQK (SEQ ID NO: 7) in the cyclic conformation compared to HHQK (SEQ ID NO: 7) in a linear compound such as a corresponding linear compound, for example as measured by ELISA or surface plasmon resonance, optionally using a method described herein.

[00124] In an embodiment, the antibody selectively binds a cyclic compound comprising the epitope sequence relative to linear peptide or a species of A-beta such as A-beta oligomer relative to monomer. In an embodiment, the selectivity is at least 2 fold, at least 3 fold, at least 5 fold, at least 10 fold, at least 20 fold, at least 30 fold, at least 40 fold, at least 50 fold, at least 100 fold, at least 500 fold, at least 1000 fold more selective for the cyclic compound and/or A-beta oligomer over a species of A-beta selected from A-beta monomer and/or A-beta fibril and/or linear HHQK (SEQ ID NO: 7), optionally linear CGHHQKG (SEQ ID NO: 12).

[00125] In an embodiment, the antibody is a monoclonal antibody. The production of monoclonals is described in the Examples.

[00126] To produce monoclonal antibodies, antibody producing cells (lymphocytes) can be harvested from a subject immunized with an immunogen described herein, and fused with myeloma cells by standard somatic cell fusion procedures thus immortalizing these cells and yielding hybridoma cells. Such techniques are well known in the art, (e.g. the hybridoma technique originally developed by Kohler and Milstein (Nature 256:495-497 (1975)) as well as other techniques such as the human B-cell hybridoma technique (Kozbor et al., Immunol.Today 4:72 (1983)), the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., Methods Enzymol, 121 : 140-67 (1986)), and screening of combinatorial antibody libraries (Huse et al., Science 246:1275 (1989)). Hybridoma cells can be screened immunochemically for production of antibodies specifically reactive with the desired epitopes and the monoclonal antibodies can be isolated.

[00127] Specific antibodies, or antibody fragments, reactive against particular antigens or molecules, may also be generated by screening expression libraries encoding immunoglobulin genes, or portions thereof, expressed in bacteria with cell surface components. For example, complete Fab fragments, VH regions and FV regions can be expressed in bacteria using phage expression libraries (see for example Ward et al., Nature 41:544-546 (1989); Huse et al., Science 246:1275-1281 (1989); and McCafferty et al., Nature 348:552-554 (1990).

[00128] In an embodiment, the antibody is a humanized antibody. As demonstrated in the Examples, specific humanized antibodies are described.

[00129] The humanization of antibodies from non-human species has been well described in the literature. See for example EP-B1 0 239400 and Carter & Merchant 1997 (Curr Opin Biotechnol 8, 449-454, 1997 incorporated by reference in their entirety herein). Humanized antibodies are also readily obtained commercially (eg. Scotgen Limited, 2 Holly Road, Twickenham, Middlesex, Great Britain.).

[00130] Humanized forms of rodent antibodies are readily generated by CDR grafting (Riechmann et al. Nature, 332:323-327, 1988). In this approach the six CDR loops comprising the antigen binding site of the rodent monoclonal antibody are linked to corresponding human framework regions. CDR grafting often yields antibodies with reduced affinity as the amino acids of the framework regions may influence antigen recognition (Foote & Winter. J Mol Biol, 224: 487-499, 1992). To maintain the affinity of the antibody, it is often necessary to replace certain framework residues by site directed mutagenesis or other recombinant techniques and may be aided by computer modeling of the antigen binding site (Co et al. J Immunol, 152: 2968-2976, 1994).

[00131] Humanized forms of antibodies are optionally obtained by resurfacing (Pedersen et al. J Mol Biol, 235: 959-973, 1994). In this approach only the surface residues of a rodent antibody are humanized.

[00132] In an embodiment, the humanized antibody comprises CDRS as shown in Table 2.

[00133] Specific humanized sequences are provided in Tables 4A and 4B.

[00134] An aspect includes a humanized antibody comprising a sequence as set forth in Table 4A or 4B or having a sequence with at least 50% sequence identity a sequence as set forth in Table 4A or 4B wherein the CDR amino acid sequences are as shown therein.

[00135] In an embodiment, the humanized antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3.

[00136] In another embodiment, the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

[00137] In another embodiment, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in any one of SEQ ID NO: 15, 17, 19, 21, 23 and 25 or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in any one of SEQ ID NO: 29, 31, 33, 35, 37 and 39 or a codon degenerate or optimized version thereof.

[00138] In another embodiment, the heavy chain variable region comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26 and/or the light chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40.

[00139] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta optionally human A-beta oligomers with an antibody comprising the heavy chain sequence as shown in Table 4A, optionally comprising a light chain sequence shown in Table 4A.

[00140] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta, optionally human A-beta oligomers with an antibody comprising the heavy chain variable chain sequence in any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26 and/or the light chain variable region sequence as set forth in SEQ ID any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40.

[00141] In an embodiment, the antibody comprises SEQ ID NO: 16 and 30; SEQ ID NO: 18 and 32; SEQ ID NO: 20 and 34; SEQ ID NO: 22 and 36; SEQ ID NO: 24 and 38; or SEQ ID NO: 36 and 40, or sequences with sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity thereto wherein the CDRs are maintained as shown in Table 2.

[00142] In another embodiment, the humanized antibody comprises a sequence as shown in Table 4B.

[00143] In an embodiment, the humanized antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54, wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NO: 74-76), or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are the sequences shown underlined therein (e.g. SEQ ID NO: 74-76).

[00144] In another embodiment, the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68, wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NOs:77-79), or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NOs:77-79).

[00145] In another embodiment, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in any one of SEQ ID NO: 43, 45, 47, 49, 51 and 53; or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in any one of SEQ ID NO: 57, 59, 61, 63, 65 and 67 or a codon degenerate or optimized version thereof.

[00146] In another embodiment, the heavy chain variable region comprises or consists of an amino acid sequence as set forth in any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54 and/or the light chain variable region comprises or consists of an amino acid sequence as set forth in SEQ ID any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68.

[00147] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the heavy chain sequence as shown in Table 4B, optionally wherein the antibody further comprises a light chain sequence shown in Table 4B.

[00148] In another embodiment, the antibody is an antibody that competes for binding to a cyclic peptide having sequence to SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the heavy chain variable chain sequence of any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54 and/or the light chain variable region sequence of any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68.

[00149] In an embodiment, the antibody comprises SEQ ID NO: 44 and 58; SEQ ID NO: 46 and 60; SEQ ID NO: 48 and 62; SEQ ID NO: 50 and 64; SEQ ID NO: 52 and 66; or SEQ ID NO: 54 and 68, or sequences with sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at

least 90% sequence identity thereto wherein the CDRs are maintained as shown underlined therein (also in in SEQ ID Nos:74-79).

[00150] In an embodiment, an antibody described herein comprises a constant region having i) an amino acid sequence as set forth in SEQ ID NO:70 and/or 72; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO:70 and/or 72; or iii) a conservatively substituted amino acid sequence i).

[00151] In another embodiment, the heavy chain constant region amino acid sequence is encoded by a nucleotide sequence as set forth in SEQ ID NO: 69; or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain constant region amino acid sequence encoded by a nucleotide sequence as set out in SEQ ID NO:71, or a codon degenerate or optimized version thereof.

[00152] Human antibodies specific to a particular antigen may be identified by a phage display strategy (Jespers et al. Bio/Technology, 12: 899-903, 1994). In one approach, the heavy chain of a rodent antibody directed against a specific antigen is cloned and paired with a repertoire of human light chains for display as Fab fragments on filamentous phage. The phage is selected by binding to antigen. The selected human light chain is subsequently paired with a repertoire of human heavy chains for display on phage, and the phage is again selected by binding to antigen. The result is a human antibody Fab fragment specific to a particular antigen. In another approach, libraries of phage are produced where members display different human antibody fragments (Fab or Fv) on their outer surfaces (Dower et al., WO 91/17271 and McCafferty et al., WO 92/01047). Phage displaying antibodies with a desired specificity are selected by affinity enrichment to a specific antigen. The human Fab or Fv fragment identified from either approach may be recloned for expression as a human antibody in mammalian cells.

[00153] Human antibodies are optionally obtained from transgenic animals (US Patent Nos. 6,150,584; 6,114,598; and 5,770,429). In this approach the heavy chain joining region (JH) gene in a chimeric or germ-line mutant mouse is deleted. Human germ-line immunoglobulin gene array is subsequently transferred to such mutant mice. The resulting transgenic mouse is then capable of generating a full repertoire of human antibodies upon antigen challenge.

[00154] Humanized antibodies are typically produced as antigen binding fragments such as Fab, Fab' F(ab')2, Fd, Fv and single domain antibody fragments, or as single chain antibodies in which the heavy and light chains are linked by a spacer. Also, the human or humanized antibodies may exist in monomeric or polymeric form. The humanized antibody optionally comprises one non-human chain and one humanized chain (i.e. one humanized heavy or light chain).

[00155] Antibodies including humanized or human antibodies are selected from any class of immunoglobulins including: IgM, IgG, IgD, IgA or IgE; and any isotype, including: IgG1, IgG2, IgG3 and IgG4. The humanized or human antibody may include sequences from one or more than one isotype or class.

[00156] Antibodies having the CDRs shown in SEQ ID Nos: 74-79 were codon optimized and made to IgG1 or IgG2a isotype. Sequences are shown in Table 8.

[00157] In an embodiment, the antibody has a sequence or a part thereof as provided in Table 8, the part comprising at least the CDRs, optionally the heavy chain CDRs and/or the light chain CDRs. In an embodiment, the part is the variable chain portion of a sequence selected from the sequences in Table 8.

[00158] The constant region shown in Table 8 (for example determinable by comparing to other sequences provided herein such as SEQ ID NOs: 42 and 56 can also be combined with the variable sequences of antibodies with CDRS having SEQ ID NOs:1-6, or 1, 2, 80, 4-6 or 1, 2, 80-83.

[00159] Additionally, antibodies specific for the epitopes described herein are readily isolated by screening antibody phage display libraries. For example, an antibody phage library is optionally screened by using a disease specific epitope of the current invention to identify antibody fragments specific for the disease specific epitope. Antibody fragments identified are optionally used to produce a variety of recombinant antibodies that are useful with different embodiments of the present invention. Antibody phage display libraries are commercially available, for example, through Xoma (Berkeley, California) Methods for screening antibody phage libraries are well known in the art.

[00160] In an embodiment, the antibody is a monoclonal antibody. In an embodiment, the antibody is a chimeric antibody such as a humanized antibody comprising the CDR sequences as recited in Table 2.

[00161] Also provided in another embodiment, is an antibody comprising CDRs as listed in Table 2 and a light chain variable region and a heavy chain variable region, optionally in the context of a single chain antibody.

[00162] The antibodies herein can be single chain antibodies. The humanized antibodies described are also in an embodiment, single chain antibodies.

[00163] As mentioned also included are antibodies that compete for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta oligomers with an antibody comprising the CDR sequences as recited in Table 2, or comprising a sequence as provided in any one of Tables 3, 4A, 4B and 8.

[00164] Competition between antibodies can be determined for example using an assay in which an antibody under test is assessed for its ability to inhibit specific binding of a reference antibody to the common antigen. A test antibody competes with a reference antibody if an excess of a test antibody (e.g., at least a 2 fold, 5, fold, 10 fold or 20 fold) inhibits binding of the reference antibody by at least 50%, at least 75%, at least 80%, at least 90% or at least 95% as measured in a competitive binding assay.

[00165] In an embodiment the antibody is isolated. In an embodiment, the antibody is an exogenous antibody.

[00166] In an embodiment, the antibody does not bind monomeric A-beta, for example under conditions described in the Examples. In an embodiment, the antibody does not bind A-beta in senile plaques, for example in situ in AD brain tissue, for example under conditions described in the Examples.

[00167] In another embodiment, the antibody does not selectively bind monomeric A-beta compared to native- or synthetic- oligomeric A-beta.

[00168] In an embodiment, the A-beta oligomer comprises A-beta 1-42 subunits.

[00169] In an embodiment, the antibody lacks A-beta fibril plaque (also referred to as senile plaque) staining, for example as measured by immuohistochemistry. Absence of plaque staining can be assessed by comparing to a positive control such as A-beta-specific antibodies 6E10 and 4G8 (Biolegend, San Diego, CA), or 2C8 (Enzo Life Sciences Inc., Farmingdale, NY) and an isotype control. An antibody described herein lacks or has negligible A-beta fibril plaque staining if the antibody does not show typical plaque morphology staining and the level of staining is comparable to or no more than 2 fold the level seen with an IgG negative isotype control. The scale can for example set the level of staining with isotype control at 1 and with 6E10 at 10. An antibody lacks A-beta fibril plaque staining if the level of staining on such a scale is 2 or less. In embodiment, the antibody shows minimal A-beta fibril plaque staining, for example on the foregoing scale, levels scored at less about or less than 3.

[00170] A further aspect is an antibody conjugated to a therapeutic, detectable label or cytotoxic agent. In an embodiment, the detectable label is a positron-emitting radionuclide. A positron-emitting radionuclide can be used for example in PET imaging.

[00171] A further aspect relates to an antibody complex comprising an antibody described herein and/or a binding fragment thereof and oligomeric A-beta.

[00172] A further aspect is an isolated nucleic acid encoding an antibody or part thereof described herein.

[00173] Nucleic acids encoding a heavy chain or a light chain or parts thereof are also provided, for example encoding a heavy chain comprising CDR-H1, CDR-H2 and/or CDR-H3 regions described herein or encoding a light chain comprising CDR-L1, CDR-L2 and/or CDR-L3 regions described herein, variable chains described herein and codon optimized and codon degenerate versions thereof.

[00174] The present disclosure also provides variants of the nucleic acid sequences that encode for the antibody and/or binding fragment thereof disclosed herein. For example, the variants include nucleotide sequences that hybridize to the nucleic acid sequences encoding the antibody and/or binding fragment thereof disclosed herein under at least moderately stringent hybridization conditions or codon degenerate or optimized sequences In another embodiment, the variant nucleic acid sequences have at least 50%, at least 60%, at least 70%, most preferably at least 80%, even

more preferably at least 90% and even most preferably at least 95% sequence identity to nucleic acid sequences encoding any of the amino acid sequences shown in Tables 2, 3, 4A, 4B and 8.

[00175] A further aspect is an isolated nucleic acid encoding an antibody described herein, for example the nucleic acids shown in any of Tables 2, 3, 4A, 4B and 8.

[00176] Another aspect is an expression cassette or vector comprising the nucleic acid herein disclosed. In an embodiment, the vector is an isolated vector.

[00177] The vector can be any vector, including vectors suitable for producing an antibody and/or binding fragment thereof or expressing a peptide sequence described herein.

[00178] The nucleic acid molecules may be incorporated in a known manner into an appropriate expression vector which ensures expression of the protein. Possible expression vectors include but are not limited to cosmids, plasmids, or modified viruses (e.g. replication defective retroviruses, adenoviruses and adeno-associated viruses). The vector should be compatible with the host cell used. The expression vectors are "suitable for transformation of a host cell", which means that the expression vectors contain a nucleic acid molecule encoding the peptides corresponding to epitopes or antibodies described herein.

[00179] In an embodiment, the vector is suitable for expressing for example single chain antibodies by gene therapy. The vector can be adapted for specific expression in neural tissue, for example using neural specific promoters and the like. In an embodiment, the vector comprises an IRES and allows for expression of a light chain variable region and a heavy chain variable region. Such vectors can be used to deliver antibody in vivo.

[00180] Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes.

[00181] Examples of such regulatory sequences include: a transcriptional promoter and enhancer or RNA polymerase binding sequence, a ribosomal binding sequence, including a translation initiation signal. Additionally, depending on the host cell chosen and the vector employed, other sequences, such as an origin of replication, additional DNA restriction sites, enhancers, and sequences conferring inducibility of transcription may be incorporated into the expression vector.

[00182] In an embodiment, the regulatory sequences direct or increase expression in neural tissue and/or cells.

[00183] In an embodiment, the vector is a viral vector.

[00184] The recombinant expression vectors may also contain a marker gene which facilitates the selection of host cells transformed, infected or transfected with a vector for expressing an antibody or epitope peptide described herein.

[00185] The recombinant expression vectors may also contain expression cassettes which encode a fusion moiety (i.e. a “fusion protein”) which provides increased expression or stability of the recombinant peptide; increased solubility of the recombinant peptide; and aid in the purification of the

target recombinant peptide by acting as a ligand in affinity purification, including for example tags and labels described herein. Further, a proteolytic cleavage site may be added to the target recombinant protein to allow separation of the recombinant protein from the fusion moiety subsequent to purification of the fusion protein. Typical fusion expression vectors include pGEX (Amrad Corp., Melbourne, Australia), pMAL (New England Biolabs, Beverly, MA) and pRIT5 (Pharmacia, Piscataway, NJ) which fuse glutathione S-transferase (GST), maltose E binding protein, or protein A, respectively, to the recombinant protein.

[00186] Systems for the transfer of genes for example into neurons and neural tissue both in vitro and in vivo include vectors based on viruses, most notably Herpes Simplex Virus, Adenovirus, Adeno-associated virus (AAV) and retroviruses including lentiviruses. Alternative approaches for gene delivery include the use of naked, plasmid DNA as well as liposome–DNA complexes. Another approach is the use of AAV plasmids in which the DNA is polycation-condensed and lipid entrapped and introduced into the brain by intracerebral gene delivery (Leone et al. US Application No. 2002076394).

[00187] Accordingly, in another aspect, the compounds, immunogens, nucleic acids, vectors and antibodies described herein may be formulated in vesicles such as liposomes, nanoparticles, and viral protein particles, for example for delivery of antibodies, compounds, immunogens and nucleic acids described herein. In particular synthetic polymer vesicles, including polymersomes, can be used to administer antibodies.

[00188] Also provided in another aspect is a cell, optionally an isolated and/or recombinant cell, expressing an antibody described herein or comprising a vector herein disclosed.

[00189] The recombinant cell can be generated using any cell suitable for producing a polypeptide, for example suitable for producing an antibody and/or binding fragment thereof. For example to introduce a nucleic acid (e.g. a vector) into a cell, the cell may be transfected, transformed or infected, depending upon the vector employed.

[00190] Suitable host cells include a wide variety of prokaryotic and eukaryotic host cells. For example, the proteins described herein may be expressed in bacterial cells such as E. coli, insect cells (using baculovirus), yeast cells or mammalian cells.

[00191] In an embodiment, the cell is a eukaryotic cell selected from a yeast, plant, worm, insect, avian, fish, reptile and mammalian cell.

[00192] In another embodiment, the mammalian cell is a myeloma cell, a spleen cell, or a hybridoma cell.

[00193] In an embodiment, the cell is a neural cell.

[00194] Yeast and fungi host cells suitable for expressing an antibody or peptide include, but are not limited to Saccharomyces cerevisiae, Schizosaccharomyces pombe, the genera Pichia or Kluyveromyces and various species of the genus Aspergillus. Examples of vectors for expression in yeast S. cerivisiae include pYepSec1, pMFa, pJRY88, and pYES2 (Invitrogen Corporation, San

Diego, CA). Protocols for the transformation of yeast and fungi are well known to those of ordinary skill in the art.

[00195] Mammalian cells that may be suitable include, among others: COS (e.g., ATCC No. CRL 1650 or 1651), BHK (e.g. ATCC No. CRL 6281), CHO (ATCC No. CCL 61), HeLa (e.g., ATCC No. CCL 2), 293 (ATCC No. 1573) and NS-1 cells. Suitable expression vectors for directing expression in mammalian cells generally include a promoter (e.g., derived from viral material such as polyoma, Adenovirus 2, cytomegalovirus and Simian Virus 40), as well as other transcriptional and translational control sequences. Examples of mammalian expression vectors include pCDM8 and pMT2PC.

[00196] In an embodiment, the cell is a fused cell such as a hybridoma cell, the hybridoma cell producing an antibody specific and/or selective for an epitope or epitope sequence described herein, including for example that selectively binds A-beta oligomers over A-beta monomers, selectively binds an epitope sequence presented in a cyclic compound relative to a linear compound or lacks or has negligible plaque binding.

[00197] A further aspect is a hybridoma cell line producing an antibody comprising the a CDR set described herein.

III. Compositions

[00198] A further aspect is a composition comprising a nucleic acid, vector or antibody described herein.

[00199] In an embodiment, the composition comprises a diluent.

[00200] Suitable diluents for nucleic acids include but are not limited to water, saline solutions and ethanol.

[00201] Suitable diluents for polypeptides, including antibodies or fragments thereof and/or cells include but are not limited to saline solutions, pH buffered solutions and glycerol solutions or other solutions suitable for freezing polypeptides and/or cells.

[00202] In an embodiment, the composition is a pharmaceutical composition comprising any of the antibodies, nucleic acids or vectors disclosed herein, and optionally comprising a pharmaceutically acceptable carrier.

[00203] The compositions described herein can be prepared by per se known methods for the preparation of pharmaceutically acceptable compositions that can be administered to subjects, optionally as a vaccine, such that an effective quantity of the active substance is combined in a mixture with a pharmaceutically acceptable vehicle.

[00204] Pharmaceutical compositions include, without limitation, lyophilized powders or aqueous or non-aqueous sterile injectable solutions or suspensions, which may further contain antioxidants, buffers, bacteriostats and solutes that render the compositions substantially compatible with the tissues or the blood of an intended recipient. Other components that may be present in such compositions include water, surfactants (such as Tween), alcohols, polyols, glycerin and vegetable

oils, for example. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules, tablets, or concentrated solutions or suspensions. The composition may be supplied, for example but not by way of limitation, as a lyophilized powder which is reconstituted with sterile water or saline prior to administration to the patient.

[00205] Pharmaceutical compositions may comprise a pharmaceutically acceptable carrier. Suitable pharmaceutically acceptable carriers include essentially chemically inert and nontoxic compositions that do not interfere with the effectiveness of the biological activity of the pharmaceutical composition. Examples of suitable pharmaceutical carriers include, but are not limited to, water, saline solutions, glycerol solutions, ethanol, N-(1(2,3-dioleyloxy)propyl)N,N,N-trimethylammonium chloride (DOTMA), diolesylphosphotidyl-ethanolamine (DOPE), and liposomes. Such compositions should contain a therapeutically effective amount of the compound, together with a suitable amount of carrier so as to provide the form for direct administration to the patient.

[00206] The composition may be in the form of a pharmaceutically acceptable salt which includes, without limitation, those formed with free amino groups such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with free carboxyl groups such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylarnino ethanol,

[00207] In an embodiment, the composition comprises an antibody described herein. In another embodiment, the composition comprises an antibody described herein and a diluent. In an embodiment, the composition is a sterile composition.

[00208] A further aspect includes an antibody complex comprising an antibody described herein and A-beta, optionally A-beta oligomer. The complex may be in solution or comprised in a tissue, optionally in vitro.

IV. Kits

[00209] A further aspect relates to a kit comprising i) an antibody and/or binding fragment thereof, ii) a nucleic acid of said antibody or a part thereof, iii) composition comprising an antibody, nucleic acid or cell described herein or iv) a recombinant cell described herein, comprised in a vial such as a sterile vial or other housing and optionally a reference agent and/or instructions for use thereof.

[00210] In an embodiment, the kit further comprises one or more of a collection vial, standard buffer and detection reagent.

[00211] In another embodiment, the kit is for diagnosing or monitoring Alzheimer's disease or a condition involving oligomeric Abeta.

V. Methods

[00212] Included are methods for making the antibodies described herein.

[00213] In particular, provided are methods of making an antibody an antibody described herein selective for a conformational epitope of HHQK (SEQ ID NO: 7) using an antibody described herein, the method comprising administering to a subject, optionally a non-human subject, a cyclic compound comprising an epitope sequence described herein, and isolating antibody producing cells or antibodies that comprise the CDRs described herein.

[00214] In an embodiment, the method is for making a monoclonal antibody using for example a method as described herein.

[00215] In another embodiment, a method of making a chimeric antibody or binding fragment thereof is provided, the method comprising using recombinant technology to subcloning a nucleic acid encoding the variable region of an antibody (heavy and/or light) described herein into a vector comprising a nucleic acid encoding a human antibody constant domain (e.g. IgG1, 2, 3, or 4), optionally with or without the Fc portion to produce a chimeric antibody vector; and expressing the chimeric antibody vector in a cell; and isolating the antibody. In an embodiment, the chimera is a mouse human chimera.

[00216] In an embodiment, the method is for making a humanized antibody using for example a method described herein. In an embodiment, the method comprises making a chimeric intermediate. The variable regions of the chimeric intermediate are for example mutagenized to introduce one or more amino acid changes outside the CDR regions. In another embodiment, one or more CDR coding sequences described herein are inserted into a human antibody scaffold.

[00217] Antibodies produced using a cyclic compound are selected as described herein and in the Examples such. In an embodiment, the method comprises isolating antibodies that specifically or selectively bind cyclic peptide over linear peptide, are specific for the epitope sequence, specifically bind oligomer and/or lack or negligibly bind plaque in situ and/or corresponding linear peptide, optionally using a method described herein.

[00218] A further aspect provides a method of detecting whether a biological sample comprises A-beta the method comprising contacting the biological sample with an antibody described herein and/or detecting the presence of any antibody complex. In an embodiment, the method is for detecting whether a biological sample comprises oligomeric A-beta.

[00219] In an embodiment, the method comprises:

a. contacting the biologic sample with an antibody described herein that is specific and/or selective for A-beta oligomer herein under conditions permissive to produce an antibody: A-beta oligomer complex; and

b. detecting the presence of any complex;

wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.

[00220] In an embodiment, the level of complex formed is compared to a test antibody such as a suitable Ig control or irrelevant antibody.

[00221] In an embodiment, the detection is quantitated and the amount of complex produced is measured. The measurement can for example be relative to a standard.

[00222] In an embodiment, the measured amount is compared to a control.

[00223] In another embodiment, the method comprises:

(a) contacting a test sample of said subject with an antibody described herein, under conditions permissive to produce an antibody-antigen complex;

(b) measuring the amount of the antibody-antigen complex in the test sample; and

(c) comparing the amount of antibody-antigen complex in the test sample to a control;

wherein detecting antibody-antigen complex in the test sample as compared to the control indicates that the sample comprises A-beta.

[00224] The control can be a sample control (e.g. from a subject without AD, or from a subject with a particular form of AD, mild, moderate or advanced), or be a previous sample from the same subject for monitoring changes in A-beta oligomer levels in the subject. Alternatively the control can be a value derived from a plurality of patients with or without AD.

[00225] In an embodiment, the antibody is an antibody having the CDR sequences described herein. In an embodiment, the antibody is a humanized antibody. In an embodiment, the antibody is a chimeric antibody.

[00226] In an embodiment, the sample is a biological sample. In an embodiment, the sample comprises brain tissue or an extract thereof and/or CSF. In an embodiment, the sample comprises whole blood, plasma or serum. In an embodiment, the sample is obtained from a human subject. In an embodiment, the subject is suspected of, at a risk of or has AD.

[00227] A number of methods can be used to detect an A-beta: antibody complex and thereby determine A-beta oligomers is present in a sample using the antibodies described herein, including immunoassays such as flow cytometry, Western blots, ELISA, SPR and immunoprecipitation followed by SDS-PAGE immunocytochemistry.

[00228] As described in the Examples surface plasmon resonance technology can be used to assess conformation specific binding. If the antibody is labeled or a detectably labeled secondary antibody specific for the complex antibody is used, the label can be detected. Commonly used reagents include fluorescent emitting and HRP labeled antibodies. In quantitative methods, the amount of signal produced can be measured by comparison to a standard or control. The measurement can also be relative.

[00229] A further aspect includes a method of measuring a level of or imaging A-beta in a subject or tissue, optionally where the A-beta to be measured or imaged is oligomeric A-beta. In an embodiment, the method comprises administering to a subject at risk or suspected of having or having AD, an antibody described herein conjugated to a detectable label; and detecting the label,

optionally quantitatively detecting the label. The label in an embodiment is a positron emitting radionuclide which can for example be used in PET imaging.

[00230] The methods may also be combined with other tests for AD or cognitive impairment. For example, synaptic protein levels, such as SNAP-25 or synaptic vesicle glycoprotein 2a (SVG2a) (Sci Transl Med. 2016 Jul 20;8(348):348ra96. doi: 10.1126/scitranslmed.aaf6667) in CSF can be measured. For example, flourodeoxyglucose PET (FDG-PET) is used as an indirect measure of synaptic metabolism.

[00231] Detecting A-beta levels using an antibody described herein can be used alone or in combination with other methods to monitor response to treatment.

[00232] It is demonstrated herein that antibodies raised against cyclo(CGHHQKG) (SEQ ID NO: 12), comprising the CDR sets described herein can specifically and/or selectively bind A-beta oligomers. Oligomeric A-beta species are believed to be the toxic propagating species in AD. Further as shown in FIG. 1 and described in the Examples, these antibodies are specific for oligomers, inhibited A-beta aggregation and A-beta oligomer propagation. Accordingly, also provided are methods of inhibiting A-beta oligomer propagation, the method comprising contacting a cell or tissue expressing A-beta with or administering to a subject in need thereof an effective amount of an A-beta oligomer specific or selective antibody described herein to inhibit A-beta aggregation and/or oligomer propagation. In vitro the assay can be monitored as described in the Examples.

[00233] The antibodies may also be useful for treating AD and/or other A-beta amyloid related diseases. For example, variants of Lewy body dementia and in inclusion body myositis (a muscle disease) exhibit similar plaques as AD and A-beta can also form aggregates implicated in cerebral amyloid angiopathy. As mentioned, the antibodies comprising the CDR sets as well as when in the humanized antibodies sequences described herein bind oligomeric A-beta which is believed to be a toxigenic species of A-beta in AD and inhibit formation of toxigenic A-beta oligomers in vitro.

[00234] Accordingly a further aspect is a method of treating AD and/or other A-beta amyloid related diseases, the method comprising administering to a subject in need thereof an effective amount of an antibody described herein comprising a CDR set described herein, optionally a humanized antibody described in Table 4A or 4B or selective or a pharmaceutical composition comprising said antibody, to a subject in need thereof. In other embodiments, nucleic acids encoding the antibodies described herein can also be administered to the subject, optionally using vectors suitable for delivering nucleic acids in a subject.

[00235] In an embodiment, a biological sample from the subject to be treated is assessed for the presence or levels of A-beta using an antibody described herein. In an embodiment, a subject with detectable A-beta levels (e.g. A-beta antibody complexes measured in vitro or measured by imaging) is treated with the antibody.

[00236] The antibody, peptides and nucleic acids can for example be comprised in a pharmaceutical composition as described herein, and formulated for example in vesicles for improving delivery.

[00237] One or more antibodies targeting HHQK (SEQ ID NO: 7) can be administered in combination. In addition the antibodies disclosed herein can be administered with one or more other treatments such as a beta-secretase inhibitor or a cholinesterase inhibitor.

[00238] Also provided are uses of the compositions, antibodies, isolated peptides, and nucleic acids for treating AD or A-beta amyloid related diseases.

[00239] The compositions, antibodies, isolated peptides and nucleic acids, vectors etc. described herein can be administered for example, by parenteral, intravenous, subcutaneous, intramuscular, intracranial, intraventricular, intrathecal, intraorbital, ophthalmic, intraspinal, intracisternal, intraperitoneal, intranasal, aerosol or oral administration.

[00240] In certain embodiments, the pharmaceutical composition is administered systemically.

[00241] In other embodiments, the pharmaceutical composition is administered directly to the brain or other portion of the CNS. For example such methods include the use of an implantable catheter and a pump, which would serve to discharge a pre-determined dose through the catheter to the infusion site. A person skilled in the art would further recognize that the catheter may be implanted by surgical techniques that permit visualization of the catheter so as to position the catheter adjacent to the desired site of administration or infusion in the brain. Such techniques are described in Elsberry et al. U.S. Patent 5,814,014 "Techniques of Treating Neurodegenerative Disorders by Brain Infusion", which is herein incorporated by reference. Also contemplated are methods such as those described in US patent application 20060129126 (Kaplitt and During "Infusion device and method for infusing material into the brain of a patient". Devices for delivering drugs to the brain and other parts of the CNS are commercially available (eg. SynchroMed® EL Infusion System; Medtronic, Minneapolis, Minnesota).

[00242] In another embodiment, the pharmaceutical composition is administered to the brain using methods such as modifying the compounds to be administered to allow receptor-mediated transport across the blood brain barrier.

[00243] Other embodiments contemplate the co-administration of the compositions, antibodies, isolated peptides and nucleic acids described herein with biologically active molecules known to facilitate the transport across the blood brain barrier.

[00244] Also contemplated in certain embodiments, are methods for administering the compositions, antibodies, isolated peptides, and nucleic acids described herein across the blood brain barrier such as those directed at transiently increasing the permeability of the blood brain barrier as described in US patent 7012061 "Method for increasing the permeability of the blood brain barrier", herein incorporated by reference.

[00245] The above disclosure generally describes the present application. A more complete understanding can be obtained by reference to the following specific examples. These examples are described solely for the purpose of illustration and are not intended to limit the scope of the application. Changes in form and substitution of equivalents are contemplated as circumstances might suggest or render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

[00246] The following non-limiting examples are illustrative of the present disclosure:

Examples

Example 1

Antibody Generation

Methods and Materials

Immunogen

[00247] Cyclo(CGHHQKG) (SEQ ID NO:12) peptide was generated at CPC Scientific, Sunnyvale, CA, USA (both cyclic and linear), and conjugated to KLH (for immunizing) and BSA (for screening) using a trifluoroacetate counter ion protocol. A linear peptide of the same sequences, CGHHQKG (SEQ ID NO: 12), were also made. Peptides were desalted and checked by MS and HPLC and deemed 95% pure. The cyclopeptide was shipped to IPA for use in production of monoclonal antibodies in mice.

Antibodies

[00248] Hybridomas and monoclonal antibodies were generated to cyclo(CGHHQKG) (SEQ ID NO: 12) linked to Keyhole Limpet Hemocyanin (KLH).

[00249] Fifty day old female BALB/c mice (Charles River Laboratories, Quebec) were immunized. A series of subcutaneous aqueous injections containing antigen but no adjuvant were given over a period of 19 days. Mice were immunized with 100µg per mouse per injection of a 0.5mg/mL cyclic peptide-KLH solution in sterile saline. All 4 mice were euthanized on Day 19 and lymphocytes were harvested for hybridoma cell line generation.

Fusion / Hybridoma Development

[00250] Lymphocytes were isolated and fused with murine SP2/0 myeloma cells in the presence of poly-ethylene glycol (PEG 1500). Fused cells were cultured using HAT selection. This method uses a semi-solid methylcellulose-based HAT selective medium to combine the hybridoma selection and cloning into one step. Single cell-derived hybridomas grow to form monoclonal colonies on the semi-solid media. 10 days after the fusion event, resulting hybridoma clones were transferred to 96-well tissue culture plates and grown in HT containing medium until mid-log growth was reached (5 days).

Hybridoma Analysis

[00251] Tissue culture supernatants from the hybridomas were tested by indirect ELISA on screening antigen (cyclic peptide-BSA) and probed for both IgG and IgM antibodies using a Goat anti-

IgG/IgM(H&L)-HRP secondary and developed with TMB substrate. Clones >0.2 OD in this assay were taken to the next round of testing. Positive cultures were retested on screening antigen to confirm secretion and on an irrelevant antigen (Human Transferrin) to eliminate non-specific mAbs and rule out false positives. Selected clones were isotyped by antibody trapping ELISA to determine if they are IgG or IgM isotype. Selected clones were also tested by indirect ELISA on other cyclic peptide-BSA conjugates as well as linear peptide-BSA conjugates to evaluate cross-reactivity and linker reactivity. Antibodies were also screened by SPR analysis.

ELISA Antibody Screening

[00252] ELISA plates were coated with 1) 0.1ug/well cyclopeptide-conjugated –BSA at 100uL/well in carbonate coating buffer (pH 9.6) O/N at 4C; 2) 0.1ug/well linear –peptide-conjugated –BSA at 100uL/well in carbonate coating buffer (pH 9.6) O/N at 4C; or 3) 0.1ug/well Negative-Peptide at 100uL/well in carbonate coating buffer (pH 9.6) O/N at 4C. Primary Antibody: Hybridoma supernatant at 100 uL/well incubated for 1 hour at 37C with shaking. Secondary Antibody 1:10,000 Goat anti-mouse IgG/IgM(H+L)-HRP at 100uL/well in PBS-Tween for 1 hour at 37C with shaking. All washing steps were performed for 30 mins with PBS-Tween. The substrate TMB was added at 50uL/well, developed in the dark and stopped with equal volume 1M HCl.

SPR Binding Assays

SPR analysis of Antibody binding to cyclic peptides, A-beta monomers and oligomers

[00253] **A-beta Monomer and Oligomer Preparation:** Recombinant A-beta40 and 42 peptides (California Peptide, Salt Lake City UT, USA) were dissolved in ice-cold hexafluoroisopropanol (HFIP). The HFIP was removed by evaporation overnight and dried in a SpeedVac centrifuge. To prepare monomers, the peptide film was reconstituted in DMSO to 5mM, diluted further to 100µM in dH2O and used immediately. Oligomers were prepared by diluting the 5mM DMSO peptide solution in phenol red-free F12 medium (Life Technologies Inc., Burlington ON, Canada) to a final concentration of 100µM and incubated for 24 hours to 7 days at 4°C.

[00254] **SPR Analysis of Cyclic Peptide, A-beta Monomer and Oligomer binding:** All SPR measurements were performed using a Molecular Affinity Screening System (MASS-1) (Sierra Sensors GmbH, Hamburg, Germany), an analytical biosensor that employs high intensity laser light and high speed optical scanning to monitor binding interactions in real time. The primary screening of tissue culture supernatants was performed using an SPR direct binding assay, whereby BSA-conjugated peptides, A-beta42 Monomer and A-beta42 Oligomer are covalently immobilized on individual flow cells of a High Amine Capacity (HAC) sensorchip (Sierra Sensors GmbH, Hamburg, Germany) and antibodies flowed over the surface. Each sample was diluted and injected in duplicate over the immobilized peptide and BSA reference surfaces, followed by injection of running buffer only for the dissociation phase. After every analytical cycle, the sensor chip surfaces were regenerated. Sensorgrams were double-referenced by subtracting out binding from the BSA reference surfaces

and blank running buffer injections, and binding response report points collected in the dissociation phase.

[00255] Protein G purified mAbs were analyzed in a secondary screen using an SPR indirect (capture) binding assay, whereby the antibodies were immobilized on a protein A-derivatized sensorchip (XanTec Bioanalytics GmbH, Duesseldorf, Germany) and A-beta40 Monomer, A-beta42 Oligomer, pooled soluble brain extracts flowed over the surface. The specificity of the antibodies was verified in an SPR direct binding assay by covalently immobilizing A-beta42 Monomer and A-beta42 Oligomer on individual flow cells of a HAC sensorchip and flowing purified mAbs over the surface.

Antibody Sequencing

[00256] The CDR and variable regions of the heavy and light chains were sequenced. Immunoglobulin gene transcripts expressed by the hybridomas were amplified from cDNA generated from the hybridoma cells using standard RT-PCR and sequenced using a standard dye-terminator capillary sequencing method.

Humanized Antibodies

[00257] Humanized Fab antibodies were prepared (Abzena) and sequenced.

Results

[00258] ELISA testing found that hybridoma clones bound the cyclopeptide preferentially over the linear peptide. Clones 301-3, 301-11 and 301-17 raised against cyclo(CGHHQKG) were selected for further analysis.

[00259] Isotyping revealed 301-3, 301-11 and 301-17 were IgG3 subtypes.

[00260] Antibodies were tested in one or more assays for their ability to bind cyclic peptide, linear peptide, A-beta 1-42 monomer and A-beta 1-42 oligomers prepared as described above.

[00261] ELISA and SPR assays confirmed that the clones preferentially bound the cylopeptide relative to the linear peptide (and were not cross reactive to unrelated cyclic peptides) and/or preferentially bound Aβ oligomers relative to monomers. The results of the binding analysis using SPR with hybridoma culture supernatants are shown in Table 1A.

[00262] Antibodies purified from the hybridoma supernatants were immobilized and assayed for their ability to bind Abeta oligomers by SPR. The results are shown in Table 1B.

Table 1A

	Cyclic Peptide(RU)	Linear Peptide(RU)	Ab42 Monomer(RU)	Ab42 Oligomer(RU)

301-11	**488**	**210.5**	**21.6**	**75.3**
301-3	**468.9**	**60.6**	**-1.8**	**56.8**

Table 1B

	Ab42 Monomer (RU)	**Ab42 Oligomer(RU)**
301-3	**-23.8**	**15.5**
301-11	**-14.1**	**-2.8**
301-17	**-27.1**	**147.8**

Antibody Sequence

[00263] Clones 301-3, 301-11 and 301-17 antibodies were sequenced. The CDR sequences of 301-3 and 301-11 are provided in Table 2. The CDRs for 301-17 are provided in SEQ ID Nos 74-79. The consensus DNA sequence and polypeptide sequences of the variable portion of the heavy and light chain of the antibodies are provided in Table 3.

[00264] As shown in Table 2, the heavy chain CDRs for 301-3 and 301-11 were identical for CDRs 1 and 2 and CDR3 varied at one position.

[00265] Two light chains were sequenced. One light chain was near identical to the light chain for 301-11.

[00266] Humanized antibodies were prepared for 301-17 and sequenced (Abzena Cambridge UK). Humanized antibody sequences are provided in Table 4A (301-11) and 4B (301-17). The CDR sequences of each antibody sequences are bolded and underlined.

Table 2

Antibody	Chain	CDR	Sequence	SEQ ID NO.
301-11	Heavy	CDR-H1	GFTFSDYY	1
301-11		CDR-H2	ISDGGSYT	2
301-11		CDR-H3	ARDYYGSSSYTSGFAY	3
301-11	Light	CDR-L1	QSLLNSRTRKNY	4
301-11		CDR-L2	WAS	5
301-11		CDR-L3	KQSYNLYT	6
301-03-1	Heavy	CDR-H1	GFTFSDYY	1
301-03-1		CDR-H2	ISDGGSYT	2
301-03 -1		CDR-H3	ARDYYGSNSYTSGFAY	80
301-03-1	Light	CDR-L1	QSLLNSRTRKNY	4
301-03-1		CDR-L2	WAS	5

301-03-1		CDR-L3	KQSYNLYT	6
301-03-2	Heavy	CDR-H1	GFTFSDYY	1
301-03-2		CDR-H2	ISDGGSYT	2
301-03-2		CDR-H3	ARDYYGSNSYTSGFAY	80
301-03-2	Light	CDR-L1	QSIVHSNGNTY	81
301-03-2		CDR-L2	KVS	82
301-03-2		CDR-L3	FQGSHVPLT	83

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Table 3

Consensus DNA sequence and translated protein sequences of the variable region. The complementarity determining regions (CDRs) are underlined according to IMTG/LIGM-DB.

Antibody and Isotype	Consensus cDNA Sequence	Polypeptide sequence
301-11 IgG3 SEQ ID NO: 8, 9	ATGAACTTTGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAA GGTGTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA GTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCT**GGA** **TTCACTTTCAGTGACTATTAC**ATGTATTGGGTTCGCCAGACTC CGGAAAAGAGGCTGGAGTGGGTCGCAACC**ATTAGTGATGGTGG** **TAGTTACACC**TCCTATCCAGACAGTGTGAAGGGACGATTCACCA TCTCCAGAGACAATGCCAAGAACAACCTGTACCTGCAAATGAGCA GTCTGAGGTCTGAGGACACAGCCATGTATTACTGT**GCAAGAGAT** **TACTACGGTAGTAGTAGCTACACCTCGGGCTTTGCTTAC**TG GGGCCAAGGGACTCTGGTCACTGTCTCTGCA	MNFGLSLIFLVLVLKG VQCEVQLVESGGGLVK PGGSLKLSCAAS**GFTF** **SDYY**MYWVRQTPEKRL EWVAT**ISDGGSYT**SY PDSVKGRFTISRDNAK NNLYLQMSSLRSEDTA MYYC**ARDYYGSSSYT** **SGFAY**WGQGTLVTVSA
301-11 Kappa SEQ ID NO: 10,11	ATGGATTCACAGGCCCAGGTTCTTATATTGCTGCTGCTATGGGTA TCTGGTACCTGTGGGGACATTGTGATGTCACAGTCTCCATCCTCC CTGGCTGTGTCAACAGGAGAGAAGGTCACTATGAGCTGCAAATCC AGT**CAGAGTCTGCTCAACAGTAGAACCCGAAAGAACTAC**TT GGCTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGAT CTAC**TGGGCATCC**ACTAGGGAATCTGGGGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTG TGCAGGCTGAAGACCTGGCAGTTTATTACTGC**AAGCAATCTTAT** **AATCTGTACACG**TTCGGAGGGGGGACCAAGCTGGAAATAAAA	MDSQAQVLILLLLWVS GTCGDIVMSQSPSSLA VSTGEKVTMSCKSS**QS** **LLNSRTRKNY**LAWYQ QKPGQSPKLLIY**WAS**T RESGVPDRFTGSGSGT DFTLTISSVQAEDLAV YYC**KQSYNLYT**FGGG TKLEIK
301-03 IgG3 SEQ ID NO: 84, 85	ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAA GGTGTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA GTGAAGCCTGGAGGGTCCCTGAAACTCTCCTGTGCAGCCTCT**GGA** **TTCACTTTCAGTGACTATTAC**ATGTATTGGGTTCGCCAGACTC CGGAAAAGAGGCTGGAGTGGGTCGCAACC**ATTAGTGATGGTGG** **TAGTTACACC**TCCTATCCAGACAGTGTGAAGGGGCGATTCACCA TCTCCAGAGACAGTGCCAAGAACAACCTGTACCTGCAAATGAGCA GTCTGAAGTCTGAGGACACAGCCATGTATTACTGT**GCAAGAGAT** **TACTACGGTAGTAATAGTTACACCTCGGGCTTTGCTTAC**TG GGGCCAAGGGACTCTGGTCACTGTCTCTGCA	MNFGLSLIFLVLVLKG VQCEVQLVESGGGLVK PGGSLKLSCAAS**GFTF** **SDYY**MYWVRQTPEKRL EWVAT**ISDGGSYT**SY PDSVKGRFTISRDSAK NNLYLQMSSLKSEDTA MYYC**ARDYYGSNSYT** **SGFAY**WGQGTLVTVSA
301-03 Kappa 1 SEQ ID NO: 86, 87	ATGGATTCACAGGCCCAGGTTCTTATATTGCTGCTGCTATGGGTA TCTGGTACCTGTGGGGACATTGTGATGTCACAGTCTCCATCCTCC CTGGCTGTGTCAGCAGGAGAGAAGGTCACTATGAGCTGCAAATCC AGT**CAGAGTCTGCTCAATAGTAGAACCCGAAAGAACTAC**TT GGCTTGGTACCAGCAGAAACCAGGGCAGTCTCCTAAACTGCTGAT CTAC**TGGGCATCC**ACTAGGGAATCTGGGGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTG TGCAGGCTGAAGACCTGGCAGTTTATTACTGC**AAGCAATCTTAT** **AATCTGTACACG**TTCGGAGGGGGGACCAAGCTGGAAATAAAA	MDSQAQVLILLLLWVS GTCGDIVMSQSPSSLA VSAGEKVTMSCKSS**QS** **LLNSRTRKNY**LAWYQ QKPGQSPKLLIY**WAS**T RESGVPDRFTGSGSGT DFTLTISSVQAEDLAV YYC**KQSYNLYT**FGGG TKLEIK
301-03	ATGAAGTTGCCTGTTAGGCTGTTGGTGCTGATGTTCTGGATTCCT	MKLPVRLLVLMFWIPA

Kappa 2 SEQ ID NO: 88, 89	GCTTCCAGCAGTGATGTTTTGATGACCCAAACTCCACTCTCCCTG CCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTGCAGATCTAGT **CAGAGCATTGTACATAGTAATGGAAACACCTAT**TTAGAATGG TACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTAC**AAA** **GTTTCC**AACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAG TGGATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGC TGAGGATCTGGGAGTTTATTTCTGC**TTTCAAGGTTCACATGTT** **CCTCTCACG**TTCGGTGCTGGGACCAAGCTGGAGCTGAAA	SSSDVLMTQTPLSLPV SLGDQASISCRSS**QSI** **VHSNGNTY**LEWYLQK PGQSPKLLIY**KVS**NRF SGVPDRFSGSGSGTDF TLKISRVEAEDLGVYF C**FQGSHVPLT**FGAGTK LELK

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Table 4A

Humanized Antibody 301-11	cDNA Sequence	Polypeptide sequence
VH0 SEQ ID NO: 13, 14	CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTTGTGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCAAGAGCAAGGCCACACTGACTCTGGACACATCCTCC AGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCT GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCGCAGGCACCACGGTCACCGTC TCCTCA	QVQLQQPGAELVKPGA SVKMSCKAS**GFTFSDY** **Y**INWVKQRPGQGLEWI GD**ISDGGSYT**YNAKFK SKATLTLDTSSSTAYM QLSSLTSEDSAVYYC**A** **RDYYGSSSYTSGFAY**W GAGTTVTVSS
VH1 SEQ ID NO: 15, 16	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGCTTAAGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCCATA AGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGACTCT GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	QVQLVQSGAELKKPGA SVKMSCKAS**GFTFSDY** **Y**INWVKQRPGQGLEWI GD**ISDGGSYT**YNAKFK SRATLTLDTSISTAYM QLSSLTSEDSAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VH2 SEQ ID NO: 17, 18	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCCATA AGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACG GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	QVQLVQSGAEVKKPGA SVKMSCKAS**GFTFSDY** **Y**INWVKQRPGQGLEWI GD**ISDGGSYT**YNAKFK SRATLTLDTSISTAYM ELSSLRSEDTAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VH3 SEQ ID NO: 19, 20	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCCATA AGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACG GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GFTFSDY** **Y**INWVRQRPGQGLEWI GD**ISDGGSYT**YNAKFK SRATLTLDTSISTAYM ELSSLRSEDTAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VH4 SEQ ID NO: 21, 22	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT	QVQLVQSGAEVKKPGA SVKVSCKAS**GFTFSDY** **Y**INWVRQRPGQGLEWI GD**ISDGGSYT**YNAKFK

	GCTAAGTTCAAGAGCAGAGTCACACTGACTCTGGACACATCCATA AGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACG GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	SRVTLTLDTSISTAYM ELSSLRSEDTAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VH5 SEQ ID NO: 23, 24	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATGGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCAAGAGCAGAGTCACACTGACTAGGGACACATCCATA AGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACG GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GFTFSDY** **Y**INWVRQRPGQGLEWM GD**ISDGGSYT**YNAKFK SRVTLTRDTSISTAYM ELSSLRSEDTAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VH6 SEQ ID NO: 25, 26	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGATTCACTTTCAGT** **GACTATTAC**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATGGGAGAT**ATTAGTGATGGTGGTAGTTACACC**TACAAT GCTAAGTTCCAGGGCAGAGTCACAATGACTAGGGACACATCCATA AGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGACACG GCGGTCTATTACTGT**GCAAGAGATTACTACGGTAGTAGTAGCTAC** **ACCTCGGGCTTTGCTTAC**TGGGGCCAAGGCACCACGGTCACCGTC TCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GFTFSDY** **Y**INWVRQRPGQGLEWM GD**ISDGGSYT**YNAKFQ GRVTMTRDTSISTAYM ELSSLRSEDTAVYYC**A** **RDYYGSSSYTSGFAY**W GQGTTVTVSS
VK0 SEQ ID NO: 27, 28	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTT GGAGATCAAGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTACCTGCAGAAA CCAGGCCAGTCTCCAAAGCTCCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCAGC GGGACCAAGCTGGAGATCAAA	DVLMTQTPLSLPVSLG DQASISCRSS**QSLLNS** **RTRKNY**LEWYLQKPGQ SPKLLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDLGVYYC**KQ** **SYNLYT**FGSGTKLEIK
VK1 SEQ ID NO: 29, 30	GATGTTTTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTTTCAGCAGAAA CCAGGCCAGTCTCCAAGGCGCCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWFQQKPGQ SPRRLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK
VK2 SEQ ID NO: 31, 32	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTTTCAGCAGAAA CCAGGCCAGTCTCCAAGGCGCCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWFQQKPGQ SPRRLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK
VK3 SEQ ID NO: 33, 34	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTTTCAGCAGAGG CCAGGCCAGTCTCCAAGGCGCCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWFQQRPGQ SPRRLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK
VK4 SEQ ID NO: 35, 36	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTACCTGCAGAGG CCAGGCCAGTCTCCAAAGCTGCTGATCTAC**TGGGCATCC**AACCGA	DVLMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWYLQRPGQ SPKLLIY**WAS**NRFSGV

	TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK
VK5 SEQ ID NO: 37, 38	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTACCAGCAGAGG CCAGGCCAGTCTCCAAGGCTGCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWYQQRPGQ SPRLLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK
VK6 SEQ ID NO: 39, 40	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGTCTGCTC** **AACAGTAGAACCCGAAAGAACTAC**TTAGAATGGTACCAGCAGAGG CCAGGCCAGTCTCCAAGGCTGCTGATCTAC**TGGGCATCC**AACCGA TTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACA GATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGA GTTTATTACTGC**AAGCAATCTTATAATCTGTACACG**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSLLNS** **RTRKNY**LEWYQQRPGQ SPRLLIY**WAS**NRFSGV PDRFSGSGSGTDFTLK ISRVEAEDVGVYYC**KQ** **SYNLYT**FGQGTKLEIK

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Table 4B

Humanized Antibody	cDNA Sequence	Polypeptide Sequence
301-17 VH0 SEQ ID NO: 41, 42 (mouse)	CAGGTCCAACTGCAGCAGCCTGGGGCTGAGCTTGTGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAAGGCCACACTGACTCTGGACACATCC TCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC TCTGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCGCAGGCACCACGGTCACCGTCTCCTCA	QVQLQQPGAELVKPGA SVKMSCKAS**GYSFTSY** **W**INWVKQRPGQGLEWI GD**VHPGRGVST**YNAKF KSKATLTLDTSSSTAY MQLSSLTSEDSAVYYC **SRSHGNTYWFFDV**WGA GTTVTVSS
VH1 SEQ ID NO: 43, 44	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGCTTAAGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC TCTGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	QVQLVQSGAELKKPGA SVKMSCKAS**GYSFTSY** **W**INWVKQRPGQGLEWI GD**VHPGRGVST**YNAKF KSRATLTLDTSISTAY MQLSSLTSEDSAVYYC **SRSHGNTYWFFDV**WGQ GTTVTVSS
VH2 SEQ ID NO: 45, 46	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGATGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGAAGCAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	QVQLVQSGAEVKKPGA SVKMSCKAS**GYSFTSY** **W**INWVKQRPGQGLEWI GD**VHPGRGVST**YNAKF KSRATLTLDTSISTAY MELSSLRSEDTAVYYC **SRSHGNTYWFFDV**WGQ GTTVTVSS
VH3 SEQ ID NO: 47, 48	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG**	QVQLVQSGAEVKKPGA SVKVSCKAS**GYSFTSY** **W**INWVRQRPGQGLEWI GD**VHPGRGVST**YNAKF KSRATLTLDTSISTAY MELSSLRSEDTAVYYC **SRSHGNTYWFFDV**WGQ

	TTTTTTGACGTCTGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	GTTVTVSS
VH4 SEQ ID NO: 49, 50	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATTGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAGAGTCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GYSFTSY** **W**INWVRQRPGQGLEWI GD**VHPGRGVST**YNAKF KSRVTLTLDTSISTAY MELSSLRSEDTAVYYC **SRSHGNTYWFFDV**WGQ GTTVTVSS
VH5 SEQ ID NO: 51, 52	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATGGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCAAGAGCAGAGTCACACTGACTAGGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GYSFTSY** **W**INWVRQRPGQGLEWM GD**VHPGRGVST**YNAKF KSRVTLTRDTSISTAY MELSSLRSEDTAVYYC **SRSHGNTYWFFDV**WGQ GTTVTVSS
VH6 SEQ ID NO: 53, 54	CAGGTCCAACTGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCCTGCAAGGCTTCT**GGCTACAGCTTCACC** **AGCTACTGG**ATAAACTGGGTGCGACAGAGGCCTGGACAAGGCCTT GAGTGGATGGGAGAT**GTGCATCCTGGTAGAGGCGTGTCCACA**TAC AATGCTAAGTTCCAGGGCAGAGTCACAATGACTAGGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT**AGCAGATCCCATGGTAACACCTACTGG** **TTTTTTGACGTC**TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA	QVQLVQSGAEVKKPGA SVKVSCKAS**GYSFTSY** **W**INWVRQRPGQGLEWM GD**VHPGRGVST**YNAKF QGRVTMTRDTSISTAY MELSSLRSEDTAVYYC **SRSHGNTYWFFDV**WGQ GTTVTVSS
VK0 SEQ ID NO: 55, 56 (mouse)	GATGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTT GGAGATCAAGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTACCTGCAGAAACCA GGCCAGTCTCCAAAGCTCCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCAGC GGGACCAAGCTGGAGATCAAA	DVLMTQTPLSLPVSLG DQASISCRSS**QSIVHS** **NGNTY**LEWYLQKPGQS PKLLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDLGVYYC**FQG** **SHVPFT**FGSGTKLEIK
VK1 SEQ ID NO: 57, 58	GATGTTTTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTTTCAGCAGAAACCA GGCCAGTCTCCAAGGCGCCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQSPLSLPVTLG QPASISCRSS**QSIVHS** **NGNTY**LEWFQQKPGQS PRRLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK
VK2 SEQ ID NO: 59, 60	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTTTCAGCAGAAACCA GGCCAGTCTCCAAGGCGCCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSIVHS** **NGNTY**LEWFQQKPGQS PRRLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK
VK3 SEQ ID NO: 61, 62	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTTTCAGCAGAGGCCA GGCCAGTCTCCAAGGCGCCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSIVHS** **NGNTY**LEWFQQRPGQS PRRLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK
VK4	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA**	DVLMTQSPLSLPVTLG QPASISCRSS**QSIVHS**

SEQ ID NO: 63, 64	**CATAGTAATGGAAACACCTAT**TTAGAATGGTACCTGCAGAGGCCA GGCCAGTCTCCAAAGCTGCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	**NGNTY**LEWYLQRPGQS PKLLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK
VK5 SEQ ID NO: 65, 66	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTACCAGCAGAGGCCA GGCCAGTCTCCAAGGCTGCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQSPLSLPVTLG QPASISCRSS**QSIVHS** **NGNTY**LEWYQQRPGQS PRLLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK
VK6 SEQ ID NO: 67, 68	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT**CAGAGCATTGTA** **CATAGTAATGGAAACACCTAT**TTAGAATGGTACCAGCAGAGGCCA GGCCAGTCTCCAAGGCTGCTGATCTAC**AAAGTTTCC**AACCGATTT TCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC**TTTCAAGGTTCACATGTTCCTTTCACT**TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQSPLSLPVTLG QPASISCRSS**QSIVHS** **NGNTY**LEWYQQRPGQS PRLLIY**KVS**NRFSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC**FQG** **SHVPFT**FGQGTKLEIK

***VH0 and VK0 are mouse sequence provided for comparison**

Table 5

Constant regions	cDNA Sequence	Polypeptide sequence
IgG4 heavy chain SEQ ID NO: 69, 70	GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCGTGGAACTCAGGCGCC CTGACCAGCGGCGTGCACACCTTCCCGGCTGTCCTACAGTCCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTCCAGCAGC TTGGGCACGAAGACCTACACCTGCAATGTAGATCACAAGCCCAGC AACACCAAGGTGGACAAGAGAGTTGAGTCCAAATATGGTCCCCCA TGCCCACCATGCCCAGCACCTGAGTTCCTGGGGGGACCATCAGTC TTCCTGTTCCCCCCAAAACCCAAGGACACTCTCATGATCTCCCGG ACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCGTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACTGGCTGAAC GGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCGTCC TCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAGCCCCGAGAG CCACAGGTGTACACCCTGCCCCCATCCCAGGAGGAGATGACCAAG AACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCCAGC GACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC TACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAAATGA	ASTKGPSVFPLAPCSR STSESTAALGCLVKDY FPEPVTVSWNSGALTS GVHTFPAVLQSSGLYS LSSVVTVPSSSLGTKT YTCNVDHKPSNTKVDK RVESKYGPPCPPCPAP EFLGGPSVFLFPPKPK DTLMISRTPEVTCVVV DVSQEDPEVQFNWYVD GVEVHNAKTKPREEQF NSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGL PSSIEKTISKAKGQPR EPQVYTLPPSQEEMTK NQVSLTCLVKGFYPSD IAVEWESNGQPENNYK TTPPVLDSDGSFFLYS RLTVDKSRWQEGNVFS CSVMHEALHNHYTQKS LSLSLGK
Kappa SEQ ID NO: 71, 72	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGAT GAGCAGTTGAAATCTGGAACTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAAC GCCCTCCAATCGGGTAACTCCCAGGAGAGTGTCACAGAGCAGGAC AGCAAGGACAGCACCTACAGCCTCAGCAGCACCCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCTTCAACAGGGGA GAGTGTTAG	RTVAAPSVFIFPPSDE QLKSGTASVVCLLNNF YPREAKVQWKVDNALQ SGNSQESVTEQDSKDS TYSLSSTLTLSKADYE KHKVYACEVTHQGLSS PVTKSFNRGEC

Example 2

Immunohistochemistry

[00283] Immunohistochemistry was performed on frozen human brain sections, with no fixation or antigen retrieval. In a humidified chamber, non-specific staining was blocked by incubation with serum-free protein blocking reagent (Dako Canada Inc., Mississauga, ON, Canada) for 1 h. The following primary antibodies were used for immunostaining: mouse monoclonal isotype controls IgG1, IgG2a, and IgG2b, and anti-amyloidβ 6E10, all purchased from Biolegend, and purified antibodies 301-11 and 301-17. All antibodies were used at 1 μg/mL. Sections were incubated at room temperature for 1h, and washed 3 x 5 min in TBS-T. Anti-Mouse IgG conjugated to Horseradish Peroxidase (1:1000) was applied to sections and incubated 45 min, then washed 3 x 5 min in TBS-T. DAB chromogen reagent (Vector Laboratories, Burlington ON, Canada) was applied and sections rinsed with distilled water when the desired level of target to background staining was achieved. Sections were counterstained with Mayer's haematoxylin, dehydrated and cover slips were applied. Slides were examined under a light microscope (Zeiss Axiovert 200M, Carl Zeiss Canada, Toronto ON, Canada) and representative images captured at 20 and 40X magnification using a Leica DC300 digital camera and software (Leica Microsystems Canada Inc., Richmond Hill, ON). Images were optimized in Adobe Photoshop using Levels Auto Correction.

[00284] **Brain Extracts**Human brain tissues were obtained from the University of Maryland Brain and Tissue Bank upon approval from the UBC Clinical Research Ethics Board (C04-0595). Clinical diagnosis of probable AD was based on NINCDS-ADRDA criteria [5].

[00285] *Homogenization*: Human brain tissue samples were weighed and subsequently submersed in a volume of fresh, ice cold TBS and EDTA-free protease inhibitor cocktail from Roche Diagnostics (Laval QC, Canada) such that the final concentration of brain tissue was 20% (w/v). Tissue was homogenized in this buffer using a mechanical probe homogenizer (3 x 30 sec pulses with 30 sec pauses in between, all performed on ice). TBS homogenized samples were then subjected to ultracentrifugation (70,000x*g* for 90 min). Supernatants were collected, aliquoted and stored at -80°C. The protein concentration of TBS homogenates was determined using a BCA protein assay (Pierce Biotechnology Inc, Rockford IL, USA).

[00286] Positive binding in brain extracts was confirmed using antibody 6E10.

SPR Analysis: 4 brain extracts from AD patients and 4 brain extracts from age-matched controls were pooled and analyzed. Brain samples, homogenized in TBS, included frontal cortex Brodmann area 9. All experiments were performed using a Molecular Affinity Screening System (MASS-1) (Sierra Sensors GmbH, Hamburg, Germany), an analytical biosensor that employs high intensity laser light and high speed optical scanning to monitor binding interactions in real time as described in Example 6. Purified antibodies generated for cyclopeptides described herein were captured on separate flow

cells of a protein A-derivatized sensor chip and diluted samples injected over the surfaces for 180 seconds, followed by 120 seconds of dissociation in buffer and surface regeneration. Binding responses were double-referenced by subtraction of mouse control IgG reference surface binding and assay buffer, and the different groups of samples compared.

Results

Brain Extracts, CSF and Immunohistochemistry

[00287] The antibodies were tested for their ability to bind A-beta in soluble brain extracts, CSF and tissue samples of cavaderic healthy control and AD brains, results are shown in Table 6. Strength of positivity in Table 6 is shown by the number plus signs.

[00288] Each of antibodies 301-11 and 301-17 showed positive binding with brain homogenates and CSF from AD patients compared to control patients.

[00289] As shown in Table 6, the purified antibodies showed preferential binding to AD vs non-AD in soluble brain extracts and CSF, and did not appreciably bind to plaque fibrils by IHC.

Table 6: Summary of binding characteristics

Antibody	Oligomers/ Monomers	Brain Extract AD/Non-AD	IHC – Plaque Staining (Frozen Section Brain 1630)	CSF
301-3	++	++	-	+
301-11	++	+++	-	++
301-17	++	++	-	+

* Scoring is relative to other clones not shown herein in the same sample category.

Example 3

Binding to A-beta synthetic oligomers.

To further verify and validate A-beta42 Oligomer binding, purified antibodies were covalently immobilized to a sensorchip, followed by the injection over the surface of commercially-prepared stable A-beta42 Oligomers (1microM) (SynAging SAS, Vandœuvre-lès-Nancy, France).

[00267] Antibodies 301-3, 301-11 and 307-17, bound the stable A-beta 42 oligomers (1 microM) with binding response units (BRUs) of an average of 14.5 (301-3), 19.3 (301-11) and 30 (301-17), respectively. By comparison, the negative control IgG1 did not meaningfully bind to the

oligomers (mean binding of BRU 2.5) while the pan-Aβ positive control antibody 6E10 bound with an average BRU of 90.

Example 4

Immunohistochemistry on Formalin Fixed Tissues

[00268] Human AD brain tissue sections were assessed using antibodies 301-11, 301-17. The patient had been previously characterized and diagnosed with Alzheimer's disease with a tripartite approach: (i) Bielschowsky silver method to demonstrate senile plaques and neurofibrillary tangles, (ii) Congo red to demonstrate amyloid and (iii) tau immunohistochemistry to demonstrate tangles and to confirm the senile plaques are "neuritic". This tissue was used to test plaque reactivity of selected monoclonal antibody clones. The brain tissues were fixed in 10% buffered formalin for several days and paraffin processed in the Sakura VIP tissue processors. Tissue sections were probed with 1μg/ml of antibody with and without microwave antigen retrieval (AR). The pan-amyloid beta reactive antibody 6E10 was included along with selected antibody clones as a positive control. Antibodies were diluted in Antibody Diluent (Ventana), color was developed with OptiView DAB (Ventana). The staining was performed on the Ventana Benchmark XT IHC stainer. Images were obtained with an Olympus BX45 microscope. Images were analyzed blind by a professional pathologist with expertise in neuropathology.

[00269] As shown in Table 7 below, using fixed tissue, the tested antibodies were negative for specific staining of senile plaque amyloid. The 6E10 antibody, used as the positive control, showed strong plaque staining.

Table 7

	Antibodies	**Staining of senile plaque amyloid**
	301-11	Neg
	301-17	Neg
Positive Control	6E10	strongly positive

Example 5

Recombinant IgG1 and IgG2a antibodies

[00270] Recombinant IgG1 and IgG2a 301-17 construct were made by grafting the CDRs of hybridoma-derived 301-17 onto a murine IgG1 or IgG2a backbone (WuXi, Biologics). The sequences are provided in Table 8.

[00271]

Table 8 – Heavy chain and light chain Sequences for 301-17 Isotypes

Antibody and Isotype	cDNA Sequence	Polypeptide sequence
301-17 IgG1 SEQ ID NO: 90, 91	CAGGTGCAGCTGCAGCAGCCTGGCGCTGAGCTGGTGAAGCCTGGA GCCTCCGTGAAGATGTCCTGCAAGGCCTCCGGCTACTCCTTCACC AGCTACTGGATCAACTGGGTGAAGCAGAGGCCCGGACAGGGCCTG GAGTGGATTGGAGACGTGCACCCTGGCCGGGGAGTGTCCACCTAC AACGCCAAGTTCAAGTCCAAGGCCACCCTGACCCTGGACACCTCC AGCTCCACCGCCTACATGCAGCTGTCCTCCCTGACCTCCGAGGAC TCCGCCGTGTACTACTGCAGCAGGTCCCACGGCAACACCTACTGG TTTTTCGACGTGTGGGGCGCCGGAACCACAGTGACCGTGTCCTCC GCCAAAACGACACCCCCATCTGTCTATCCACTGGCCCCTGGATCT GCTGCCCAAACTAACTCCATGGTGACCCTGGGATGCCTGGTCAAG GGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCCTGGAGTCTGAC CTCTACACTCTGAGCAGCTCAGTGACTGTCCCCTCCAGCCCTCGG CCCAGCGAGACCGTCACCTGCAACGTTGCCCACCCGGCCAGCAGC ACCAAGGTGGACAAGAAAATTGTGCCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCCAGAAGTATCATCTGTCTTCATCTTC CCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAG GTCACGTGTGTTGTGGTAGACATCAGCAAGGATGATCCCGAGGTC CAGTTCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAG ACGCAACCCCGGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTC AGTGAACTTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAG TTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCCATCGAG AAAACCATCTCCAAAACCAAAGGCAGACCGAAGGCTCCACAGGTG TACACCATTCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTC AGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACT GTGGAGTGGCAGTGGAATGGGCAGCCAGCGGAGAACTACAAGAAC ACTCAGCCCATCATGAACACGAATGGCTCTTACTTCGTCTACAGC AAGCTCAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTC ACCTGCTCTGTGTTACATGAGGGCCTGCACAACCACCATACTGAG AAGAGCCTCTCCCACTCTCCTGGTAAATGATGA	QVQLQQPGAELVKPGA SVKMSCKASGYSFTSY WINWVKQRPGQGLEWI GDVHPGRGVSTYNAKF KSKATLTLDTSSSTAY MQLSSLTSEDSAVYYC SRSHGNTYWFFDVWGA GTTVTVSSAKTTPPSV YPLAPGSAAQTNSMVT LGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAV LESDLYTLSSSVTVPS SPRPSETVTCNVAHPA SSTKVDKKIVPRDCGC KPCICTVPEVSSVFIF PPKPKDVLTITLTPKV TCVVVDISKDDPEVQF SWFVDDVEVHTAQTQP REEQFNSTFRSVSELP IMHQDWLNGKEFKCRV NSAAFPAPIEKTISKT KGRPKAPQVYTIPPPK EQMAKDKVSLTCMITD FFPEDITVEWQWNGQP AENYKNTQPIMNTNGS YFVYSKLNVQKSNWEA GNTFTCSVLHEGLHNH HTEKSLSHSPGK
301-17 IgG2a SEQ ID NO: 92, 93	CAGGTGCAGCTGCAGCAGCCTGGCGCTGAGCTGGTGAAGCCTGGA GCCTCCGTGAAGATGTCCTGCAAGGCCTCCGGCTACTCCTTCACC AGCTACTGGATCAACTGGGTGAAGCAGAGGCCCGGACAGGGCCTG GAGTGGATTGGAGACGTGCACCCTGGCCGGGGAGTGTCCACCTAC AACGCCAAGTTCAAGTCCAAGGCCACCCTGACCCTGGACACCTCC AGCTCCACCGCCTACATGCAGCTGTCCTCCCTGACCTCCGAGGAC TCCGCCGTGTACTACTGCAGCAGGTCCCACGGCAACACCTACTGG TTTTTCGACGTGTGGGGCGCCGGAACCACAGTGACCGTGTCCTCC GCCAAAACAACAGCCCCATCGGTCTATCCACTGGCCCCTGTGTGT GGAGATACAACTGGCTCCTCGGTGACTCTAGGATGCCTGGTCAAG GGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACTCTGGATCC CTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCCTGCAGTCTGAC CTCTACACCCTCAGCAGCTCAGTGACTGTAACCTCGAGCACCTGG CCCAGCCAGTCCATCACCTGCAATGTGGCCCACCCGGCAAGCAGC ACCAAGGTGGACAAGAAAATTGAGCCCAGAGGGCCCACAATCAAG CCCTGTCCTCCATGCAAATGCCCAGCACCTAACCTCTTGGGTGGA CCATCCGTCTTCATCTTCCCTCCAAAGATCAAGGATGTACTCATG ATCTCCCTGAGCCCCATAGTCACATGTGTGGTGGTGGATGTGAGC GAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTG GAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAAC AGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGAC TGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAAGAC CTCCCAGCGCCCATCGAGAGAACCATCTCAAAACCCAAAGGGTCA GTAAGAGCTCCACAGGTATATGTCTTGCCTCCACCAGAAGAAGAG ATGACTAAGAAACAGGTCACTCTGACCTGCATGGTCACAGACTTC	QVQLQQPGAELVKPGA SVKMSCKASGYSFTSY WINWVKQRPGQGLEWI GDVHPGRGVSTYNAKF KSKATLTLDTSSSTAY MQLSSLTSEDSAVYYC SRSHGNTYWFFDVWGA GTTVTVSSAKTTAPSV YPLAPVCGDTTGSSVT LGCLVKGYFPEPVTLT WNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTS STWPSQSITCNVAHPA SSTKVDKKIEPRGPTI KPCPPCKCPAPNLLGG PSVFIFPPKIKDVLMI SLSPIVTCVVVDVSED DPDVQISWFVNNVEVH TAQTQTHREDYNSTLR VVSALPIQHQDWMSGK EFKCKVNNKDLPAPIE RTISKPKGSVRAPQVY VLPPPEEEMTKKQVTL TCMVTDFMPEDIYVEW TNNGKTELNYKNTEPV

	ATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACA GAGCTAAACTACAAGAACACTGAACCAGTCCTGGACTCTGATGGT TCTTACTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGG GTGGAAAGAAATAGCTACTCCTGTTCAGTGGTCCACGAGGGTCTG CACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAA TGATGA	LDSDGSYFMYSKLRVE KKNWVERNSYSCSVVH EGLHNHHTTKSFSRTP GK
301-17 Kappa SEQ ID NO: 94, 95	GATGTGCTGATGACCCAGACCCCTCTGTCCCTGCCTGTGTCCCTG GGCGATCAGGCCAGCATCTCCTGCAGGTCCTCCCAGTCCATCGTG CACTCCAACGGCAACACCTACCTGGAGTGGTACCTGCAGAAGCCC GGCCAGTCCCCCAAGCTGCTGATCTACAAGGTGTCCAACCGGTTC TCCGGCGTGCCCGATAGGTTCTCCGGATCCGGCTCCGGCACCGAC TTTACCCTGAAGATCTCCAGGGTGGAGGCCGAGGACCTGGGCGTG TACTACTGCTTTCAGGGCTCCCACGTGCCCTTCACCTTCGGCTCC GGCACCAAGCTGGAGATCAAGCGGGCTGATGCTGCACCAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCC TCAGTCGTGTGCTTCTTGAACAACTTCTACCCCAAAGACATCAAT GTCAAGTGGAAGATTGATGGCAGTGAACGACAAAATGGCGTCCTG AACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATG AGCAGCACCCTCACGTTGACCAAGGACGAGTATGAACGACATAAC AGCTATACCTGTGAGGCCACTCACAAGACATCAACTTCACCCATT GTCAAGAGCTTCAACAGGAATGAGTGTTGATGA	DVLMTQTPLSLPVSLG DQASISCRSSQSIVHS NGNTYLEWYLQKPGQS PKLLIYKVSNRFSGVP DRFSGSGSGTDFTLKI SRVEAEDLGVYYCFQG SHVPFTFGSGTKLEIK RADAAPTVSIFPPSSE QLTSGGASVVCFLNNF YPKDINVKWKIDGSER QNGVLNSWTDQDSKDS TYSMSSTLTLTKDEYE RHNSYTCEATHKTSTS PIVKSFNRNEC

[00272] The 301-17 IgG1 and IgG2a antibodies were tested and compared to the parent hybridoma-purified IgG3 antibody for binding characteristics as described below.

[00273] *301-17 IgG2a ProteOn Biosensor (BioRad) Binding to AbO:* Recombinant 301-17 IgG2a and hybridoma-purified 301-17 IgG3 were captured with anti-mouse IgG or amine coupling on Proteon GLM Sensor chips and tested for AbO binding (SynAging AbO). AbO 3 fold-dilutions were used: 1 uM, 0.33 uM, 0.11 uM, 37 nM, 12.3 nM . Assay buffer was PBS-E + Tween 20 + 2 mg/ml BSA.

Results:

[00274] Approximate kinetic values were:

Hybridoma: KD = 26.9 nM

IgG2a-301-17 antibody: KD = 16.2 - 19.5 nM

No binding was detected with control mouse IgG.

[00275] 301-17 IgG2a *ProteOn Biosensor (BioRad) Binding to cyclic peptide epitope:* Recombinant 301-17 IgG2a was amine-coupled to Proteon GLH biosensor chip and tested for binding to cyclopeptide of SEQ ID NO: 2 coupled to BSA. Cyclo-BSA 3-fold dilutions were used from 9 nM to 111 pM. Assay buffer was PBS-E + 0.05% Tween + 10 mg/ml BSA. Antibody 301-17 IgG2a was found to bind cyclic peptide (SEQ ID NO: 12) conjugated to BSA with an approximate KD of 17 pM (average of 3 tests). No or negligible binding was detected for other commercial Abeta antibodies tested (pan-Abeta 6E10, Biolegend) and rabbit anti-Abeta antibodies (D54D2, Cell Signaling; ab201060, (abcam; NBP1-78007, Novus).

[00276] *301-17 IgG1 MAAS-2 binding to AbO*: Recombinant 301-17 IgG1 and hybridoma-purified 301-17 IgG3 were immobilized on MAAS-2 sensor chips and tested for binding to AbO (SynAging) at 1 uM. Under the conditions tested, the recombinant IgG1 301-17 antibody gave a

greater signal than the hybridoma -purified antibody in 2 tests (40-55 BRU vs 15-25 BRU, respectively). Little or no binding was detected with control mouse IgG.

[00277] *301-17 IgG1 MAAS-2 binding to cyclic peptide epitope:* Recombinant 301-17 IgG1 was immobilized on MAAS-2 sensor chip and tested for binding to cyclopeptide of SEQ ID NO: 2 coupled to BSA at pH 6.5, 7.5 or 8.0. Equivalently high levels of binding were observed for 301-17 IgG1 under all 3 pH conditions (~400 BRUs). Little or no binding was detected under any of the pH conditions for control mouse IgG or the pan-Abeta 6E10 antibody (Biolegend)

Example 6

Inhibition of Oligomer Propagation

[00278] The biological functionality of antibodies was tested in vitro by examining their effects on Amyloid Beta (Aβ) aggregation using the Thioflavin T (ThT) binding assay. Aβ aggregation is induced by and propagated through nuclei of preformed small Aβ oligomers, and the complete process from monomeric Aβ to soluble oligomers to insoluble fibrils is accompanied by concomitantly increasing beta sheet formation. This can be monitored by ThT, a benzothiazole salt, whose excitation and emission maxima shifts from 385 to 450nm and from 445 to 482nm respectively when bound to beta sheet-rich structures and resulting in increased fluorescence. Briefly, Aβ 1-42 (Bachem Americas Inc., Torrance, CA) was solubilized, sonicated, diluted in Tris-EDTA buffer (pH7.4) and added to wells of a black 96-well microtitre plate (Greiner Bio-One, Monroe, NC) to which equal volumes of cyclopeptide raised antibody or irrelevant mouse IgG antibody isotype controls were added, resulting in a 1:5 molar ratio of Aβ1-42 peptide to antibody. ThT was added and plates incubated at room temperature for 24 hours, with ThT fluorescence measurements (excitation at 440nm, emission at 486nm) recorded every hour using a Wallac Victor3v 1420 Multilabel Counter (PerkinElmer, Waltham, MA). Fluorescent readings from background buffer were subtracted from all wells, and readings from antibody only wells were further subtracted from the corresponding wells.

[00279] Aβ42 aggregation, as monitored by ThT fluorescence, demonstrated a sigmoidal shape characterized by an initial lag phase with minimal fluorescence, an exponential phase with a rapid increase in fluorescence and finally a plateau phase during which the Aβ molecular species are at equilibrium and during which there is no increase in fluorescence. Co-incubation of Aβ42 with an irrelevant mouse antibody did not have any significant effect on the aggregation process. In contrast, co-incubation of Aβ42 with the test antibodies completely inhibited all phases of the aggregation process. Results obtained with antibody 301-11 are shown in FIG. 1.

[00280] Near identical results were obtained with 301-17 as well as 301-3.

[00281] As the ThT aggregation assay mimics the in vivo biophysical / biochemical stages of Aβ propagation and aggregation from monomers, oligomers, protofibrils and fibrils that is pivotal in AD

pathogenesis, the antibodies demonstrate the potential to completely abrogate this process. Isotype control performed using mouse IgG control antibody showed no inhibition.

Example 7

Toxicity inhibition assay

[00282] The inhibition of toxicity of A-beta42 oligomers by antibodies can be tested in a rat primary cortical neuron assay.

[00283] Antibody and control IgG are each adjusted to a concentration such as 2 mg/mL. Various molar ratios of A-beta oligomer and antibody are tested along with a vehicle control, A-beta oligomer alone and a positive control such as the neuroprotective peptide humanin HNG.

[00284] An exemplary set up is shown in Table 9.

[00285] Following preincubation for 10 minutes at room temperature, the volume is adjusted to 840 microlitres with culture medium. The solution is incubated for 5 min at 37C. The solution is then added directly to the primary cortical neurons and cells are incubated for 24h. Cell viability can be determined using the MTT assay.

Table 9

AβO / MAB molar ratio	AβO (µL)	AβO (µM)	AB (µM)	AB (µL)	Medium (µL)	Final volume (µL)
5/1	1.68	4.2	0.84	12.73	185.6	200
1/1	1.68	4.2	4.20	63.64	134.7	200
1/2	1.68	4.2	8.4	127.27	71.1	200

AβO working solution: 2,2 mg/mL - 500 µM

CTRL vehicle:	1,68 µL of oligomer buffer + 127,3 µL PBS + 711 µL culture medium
CTRL AβO:	1,68 µL of AβO + 127,3 µL PBS + 711 µL culture medium
CTRL HNG:	1,68 µL of AβO + 8,4 µL HNG (100 nM final) + 127,3 µL PBS + 702,6 µL culture medium

[00286] In the absence of A-beta oligomers, the 301-17 antibody alone had no effect on neuronal cell viability. When incubated in the presence of A-beta oligomers, the antibody inhibited A-beta oligomer-induced neuronal death at all molar ratios tested

Example 8

In vivo toxicity inhibition assay

[00287] The inhibition of toxicity of A-beta42 oligomers by the antibodies can be tested in vivo in mouse behavioral assays.

Novel Object Recognition (NOR)

[00288] The Novel Object Recognition (NOR) model utilizes the normal behavior of rodents to investigate novel objects for a significantly longer time than known objects. This test assesses recognition memory for items and its human equivalent is the visual pairwise-comparison (VPC). Recognition of objects is mediated by the perirhinal cortex in rodents, primates and humans. AD pathology develops first in the perirhinal and enthorinal cortex before the hippocampus. The VPC task detects memory deficit in mild cognitive impairment (MCI) and conversion from MCI to AD is predicted by this task (16).

Results:

[00289] The assay was performed by (SynAging SAS, Vandœuvre-lès-Nancy, France). Twelve C57BL6J mice per group (11-12 weeks old) were ICV-injected with vehicle (buffer used for Aβ oligomerization) or AβO (50 pmoles) in the presence of vehicle (PBS) or antibody 301-17 on day 0. The cognitive performance of all mice was determined by a Novel Object Recognition (NOR) test performed at days +7 and +8.

[00290] The study, done in blind to the operators, involved a total of 48 mice divided in four experimental groups with 12 mice per experimental group. All animals received a single (and unilateral) ICV injection of vehicle OR AβO in the absence or presence of antibody in a total volume of 5 µL. The experimental groups were defined as follow:

- GROUP A (vehicle CTRL): ICV injection of vehicle (n = 12)
- GROUP B (AβO CTRL): ICV injection of AβO (n = 12)
- GROUP C (Antibody CTRL): ICV injection of AβO + antibody (n = 12)
- GROUP D (Treatment): ICV injection of AβO + antibody (n = 12)

[00291] Before ICV injection, 4 µL of antibody 1 (*i.e.* 112 pmoles) were incubated for 30 minutes at room temperature with 1 µL vehicle (*i.e.* buffer for Aβ oligomerization) or 1 µL AβO (50 pmoles) corresponding to an antibody/AβO molar ratio of 2.24.

[00292] At day 0, mice received a single 5 µL ICV injection of vehicle or AβO in the presence of vehicle or antibody.

[00293] The NOR test was conducted in one trial with all 48 mice at days +7 and +8. One day before the cognitive test (*i.e.* at Day +7), mice are habituated during a 10 min trial during which they are placed in an empty open field. The day of the cognitive test (*i.e.* Day +8), animals are placed in the same open field and are allowed to explore freely two identical objects for a trial of five minutes (acquisition trial). Then the animals are returned to their home cage for an inter-trial time of five minutes. During the retention trial, animals are allowed to explore two different objects: the same familiar object and one novel object. During this time, the experimenter, blind to the treatment, records

the time the mouse is actively exploring each object. All trials are video recorded (Smart v3.0 software, Bioseb). A discrimination index (DI) is then generated: (DI) = (time exploring novel object – time exploring familiar object) / total exploration time. If the total exploration time is ≤ 5 s, animals are excluded from the calculation of the discrimination index and statistical analysis.

[00294] Mice from the vehicle control group (Group A) exhibited normal behavior with a mean discrimination index of 0.443 ± 0.053. These results are in agreement with previous observations of similar control groups at SynAging. As expected, a single ICV injection of AβO (Group B) resulted in a significant impairment ($p<0.0001$) of the cognitive performance when compared to vehicle control mice; with a mean discrimination index of -0.062 ± 0.048. AβO-injected mice were not able to discriminate between novel and familiar objects.

[00295] Mice dosed with antibody in the presence of vehicle (Group C) were found to exhibit normal cognitive performances with a mean discrimination index of 0.439 ± 0.049. These mice were not significantly different from vehicle control mice ($p=0.9163$) and significantly different from AβO injected mice ($p<0.0001$).

[00296] When co-injected with AβO, the antibody fully prevented AβO-induced cognitive deficits in the NOR test. Indeed, mice from Group D exhibited a mean discrimination index of 0.481 ± 0.055, not different from control mice ($p=0.6126$) but different from AβO-injected mice ($p=0.0002$) . Taken together, the data suggest that antibody 301-17 offered protection against AβO-induced cognitive deficits.

Synaptic Markers

[00297] In addition to behavioral assays, brain tissue can be collected and analyzed for levels of synaptic markers (PSD95, SNAP25, synaptophysin) and inflammation markers (IL-1-beta and TNF-alpha). Mice are sacrificed at ~14 days post-ICV injection of oligomers and perfused with saline. Hippocampi are collected, snap frozen and stored at -80^{0}C until analyzed. Protein concentrations of homogenized samples are determined by BCA. Concentration of synaptic markers are determined using ELISA kits (Cloud-Clone Corp, USA). Typically, synaptic markers are reduced by 25-30% in mice injected with A-beta oligomers and restored to 90-100% by the humanin positive control. Concentrations of the IL-1-beta inflammatory markers are increased approximately 3-fold in mice injected with A-beta oligomers and this increase is largely prevented by humanin.

[00298] Brains are collected from mice that underwent the behavioral testing.

[00299] The hippocampus (relevant structure for memory formation) is dissected and homogenized in RIPA buffer containing an anti-protease cocktail. The tissue is lysed by 3 freeze thaw cycles carried out in liquid nitrogen and a water bath at 37C. and the supernatants are recovered after centrifuging.

[00300] The lysate can be analyzed for levels of TNF-alpha (increases with inflammation) and levels of the synaptic markers PSD-95 and SNAP-25 (which go down when there is synaptic damage).

[00301] The antibody showed complete protection in the behavioral assay. It is expected that brains will also show an improvement in both SNAP25 and PSD-95 levels and a decrease in TNF-alpha levels in the brain.

[00302]

Example 9

In vivo propagation inhibition assay

[00303] In vivo propagation of A-beta toxic oligomers and associated pathology can be studied in various rodent models of Alzheimer's disease (AD). For example, mice transgenic for human APP (e.g. APP23 mice) or human APP and PSEN1 (APPPS1 mice) express elevated levels of A-beta and exhibit gradual amyloid deposition with age accompanied by inflammation and neuronal damage. Intracerebral inoculation of oligomer-containing brain extracts can significantly accelerate this process (13, 14). These models provide a system to study inhibition of A-beta oligomer propagation by test antibodies administered intracerebrally or systemically.

Table 10 A-beta Sequences and compounds

1)
HHQK (SEQ ID NO: 7)
CGHHQKG, cyclo(CGHHQKG) (SEQ ID NO: 12)

Table 11

Human A-beta 1-42

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 73)

[00304] While the present application has been described with reference to what are presently considered to be the preferred examples, it is to be understood that the application is not limited to the disclosed examples. To the contrary, the application is intended to cover various modifications and equivalent arrangements included within the spirit and scope of the appended claims.

[00305] All publications, patents and patent applications are herein incorporated by reference in their entirety to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Specifically, the sequences associated with each accession numbers provided herein including for example accession numbers and/or biomarker sequences (e.g. protein and/or nucleic acid) provided in the Tables or elsewhere, are incorporated by reference in its entirely.

[00306] The scope of the claims should not be limited by the preferred embodiments and examples, but should be given the broadest interpretation consistent with the description as a whole.

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Claims:

1. An isolated antibody comprising a light chain variable region and a heavy chain variable region, optionally fused, the heavy chain variable region comprising complementarity determining regions CDR-H1, CDR-H2 and CDR-H3, the light chain variable region comprising complementarity determining region CDR-L1, CDR-L2 and CDR-L3 and with the amino acid sequences of said CDRs comprising or consisting of the sequences SEQ ID Nos: 1-6; or SEQ ID Nos:1,2, 80 and 4-6, or SEQ ID Nos: 1, 2, 80-83.

2. The antibody of claim 1 wherein the amino acid sequences of said CDRs comprise or consist of the sequences:

CDR-H1 GFTFSDYY	(SEQ ID NO: 1)
CDR-H2 ISDGGSYT	(SEQ ID NO: 2)
CDR-H3 ARDYYGSSSYTSGFAY	(SEQ ID NO: 3)
CDR-L1 QSLLNSRTRKNY	(SEQ ID NO: 4)
CDR-L2 WAS	(SEQ ID NO: 5)
CDR-L3 KQSYNLYT	(SEQ ID NO: 6).

3. The antibody of claim 2, wherein the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 9; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 9, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3.

4. The antibody of claim 2 or 3, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 11, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 11, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

5. The antibody of any one of claims 2 to 4, wherein the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or to human A-beta optionally human A-beta oligomers, oligomers with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1-6.

6. The antibody of claim 1 with the amino acid sequences of said CDRs comprising or consisting of the sequences:

CDR-H1 GFTFSDYY (SEQ ID NO: 1)

CDR-H2 ISDGGSYT (SEQ ID NO: 2)

CDR-H3 ARDYYGSNSYTSGFAY (SEQ ID NO: 80)

CDR-L1 QSLLNSRTRKNY (SEQ ID NO: 4)

CDR-L2 WAS (SEQ ID NO: 5)

CDR-L3 KQSYNLYT (SEQ ID NO: 6).

7. The antibody of claim 6, wherein the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 85; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 85, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80.

8. The antibody of claim 6 or 7, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 87, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 89, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

9. The antibody of any one of claims 6 to 8, wherein the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta optionally human A-beta oligomers, with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1, 2, 80, 4-6.

10. The antibody of claim 1 with the amino acid sequences of said CDRs comprising or consisting of the sequences:

CDR-H1 GFTFSDYY (SEQ ID NO: 1)

CDR-H2 ISDGGSYT (SEQ ID NO: 2)

CDR-H3 ARDYYGSNSYTSGFAY (SEQ ID NO: 80)

CDR-L1 QSIVHSNGNTY (SEQ ID NO: 81)

CDR-L2 KVS (SEQ ID NO: 82)

CDR-L3 FQGSHVPLT (SEQ ID NO: 83).

11. The antibody of claim 10, wherein the antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in SEQ ID NO: 85; ii) an amino acid sequence with

at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 85, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 80.

12. The antibody of claim 10 or 11, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth in SEQ ID NO: 89, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to SEQ ID NO: 89, wherein the CDR sequences are as set forth in SEQ ID NO: 81, 82 and 83, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 81, 82 and 83.

13. The antibody of any one of claims 10 to 12, wherein the antibody is an antibody that competes for binding to a cyclic peptide having sequence of SEQ ID NO: 12, and/or human A-beta optionally human A-beta oligomers, with an antibody comprising the CDR sequences as recited herein in SEQ ID Nos: 1, 2, 80-3.

14. The antibody of any one of claims 1 to 13, wherein the antibody wherein the antibody selectively binds to a cyclic compound comprising HHQK (SEQ ID NO: 7) over a corresponding linear peptide.

15. The antibody of any one of claims 1 to 14, wherein the antibody selectively binds A-beta oligomer over A-beta monomer and/or A-beta fibril.

16. The antibody of any one of claims 1 to 15, wherein the antibody lacks or has negligible binding to A-beta monomer and/or A-beta fibril plaques in situ.

17. The antibody of any one of claims 1 to 16 wherein the antibody is a monoclonal antibody.

18. The antibody of any one of claims 1 to 17, wherein the antibody is a humanized antibody.

19. The antibody of any one of claims 1 to 17, wherein the antibody is a human antibody.

20. The antibody of any one of claims 1 to 18, wherein the antibody is an antibody binding fragment selected from Fab, Fab', F(ab')2, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies, diabodies, and multimers thereof.

21. The antibody of claim 1, wherein the isotype is IgG1, IgG2 or IgG4.

22. The antibody of claim 21, wherein the isotype is IgG2, optionally IgG2a.

23. The antibody of any one of claims 1 to 22, wherein the antibody comprises a sequence or a part thereof selected from a sequence set forth in Table 8 wherein the part comprises at least CDRs 1-3, optionally heavy chain CDRs and/or light chain CDRs.

24. The antibody of claim 23, wherein the part is the variable domain nucleic acid sequence.

25. A humanized antibody comprising a sequence as set forth in Table 4A or 4B or a sequence with at least 50% sequence identity thereto wherein the CDR amino acid sequences are as set for forth in SEQ ID NO: 1-6; SEQ ID Nos: 1, 2, 80, 4-6; SEQ IDS Nos: 1, 2, 80-83 or SEQ ID Nos: 74-79.

26. The humanized antibody of claim 25, wherein the humanized antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 16, 18, 20, 22, 24 and 26, wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3, or iii) a conservatively substituted amino acid sequence i) wherein the CDR sequences are as set forth in SEQ ID NO: 1, 2 and 3.

27. The humanized antibody of claim 25 or 26, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 30, 32, 34, 36, 38 and 40, wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6, or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 4, 5 and 6.

28. The humanized antibody of any one of claims 25 to 27, the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in any one of SEQ ID NO: 15, 17, 19, 21, 23 and 25 or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in any one of SEQ ID NO: 29, 31, 33, 35, 37 and 39 or a codon degenerate or optimized version thereof.

29. The humanized antibody of claim 25, wherein the antibody comprises SEQ ID NO: 16 and 30; SEQ ID NO: 18 and 32; SEQ ID NO: 20 and 34; SEQ ID NO: 22 and 36; SEQ ID NO: 24 and 38; or SEQ ID NO: 36 and 40, or sequences with sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity thereto wherein the CDRs are maintained as shown in Table 2.

30. The humanized antibody of claim 25, wherein the humanized antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54; ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 44, 46, 48, 50, 52 and 54, wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NO: 74-76), or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are the sequences shown underlined therein (e.g. SEQ ID NO: 74-76).

31. The humanized antibody of claim 25 or 30, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68, ii) an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity to any one of SEQ ID NO: 58, 60, 62, 64, 66 and 68, wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NOs:77-79), or iii) a conservatively substituted amino acid sequence of i) wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NOs:77-79).

32. The humanized antibody of claim 25, 30 or 31, wherein the heavy chain variable region amino acid sequence is encoded by a nucleotide sequence as set forth in any one of SEQ ID NO: 43, 45, 47, 49, 51 and 53; or a codon degenerate or optimized version thereof; and/or the antibody comprises a light chain variable region amino acid sequence encoded by a nucleotide sequence as set out in any one of SEQ ID NO: 57, 59, 61, 63, 65 and 67 or a codon degenerate or optimized version thereof.

33. The humanized antibody of any one of claims 25, 29, 30 or 31, wherein the antibody comprises SEQ ID NO: 44 and 58; SEQ ID NO: 46 and 60; SEQ ID NO: 48 and 62; SEQ ID NO: 50 and 64; SEQ ID NO: 52 and 66; or SEQ ID NO: 54 and 68, or sequences with sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% sequence identity thereto wherein the CDRs are maintained as shown underlined therein (also in in SEQ ID Nos:74-79).

34. The humanized antibody of claim 25, wherein the humanized antibody has a heavy chain variable sequence and a light chain variable sequence as shown in Table 4A or 4B.

35. The antibody of any one of claims 1 to 34, wherein the antibody is a single chain antibody.

36. An immunoconjugate comprising the antibody of any one of claims 1 to 35 and a detectable label or cytotoxic agent.

37. The immunoconjugate of claim 36, wherein the detectable label comprises a positron emitting radionuclide, optionally for use in subject imaging such as PET imaging.

38. A composition comprising the antibody of any one of claims 1 to 35, or the immunoconjugate of claim 36 or 37, optionally with a diluent.

39. A nucleic acid molecule encoding the antibody of any one of claims 1 to 35.

40. A vector comprising the nucleic acid of claim 39.

41. A cell expressing an antibody of any one of claims 1 to 35, optionally wherein the cell is a hybridoma comprising the vector of claim 39.

42. A kit comprising the antibody of any one of claims 1 to 35, , the nucleic acid molecule of claim 39, the vector of claim 40 or the cell of claim 41.

43. A method of making an antibody of claim 1 comprising administering a cyclopeptide having an A-beta sequence of SEQ ID NO: 12 or a composition comprising said cyclopeptide to a subject, such as a non-human mammal, and isolating antibody and/or cells expressing antibody which has CDRs or competes with an antibody comprising or consisting of the CDRs selected from SEQ ID Nos in Table 2.

44. A method of determining if a biological sample comprises A-beta, the method comprising:

a. contacting the biological sample with an antibody of any one of claims 1 to 35 or the immunoconjugate of claim 36 or 37; and

b. detecting the presence of any antibody complex.

45. The method of claim 44 for determining if the biological sample contains A-beta oligomer the method comprising:

a. contacting the sample with the antibody of any one of claims 1 to 35 or the immunoconjugate of claim 36 or 37 that is specific and/or selective for A-beta oligomers under conditions permissive for forming an antibody: A-beta oligomer complex; and

b. detecting the presence of any complex;

wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.

46. The method of claim 45, wherein the amount of complex is measured.

47. The method of any one of claims 44 to 46, wherein the sample comprises brain tissue or an extract thereof, whole blood, plasma, serum and/or CSF.

48. The method of any one of claims 44 to 47, wherein the sample is compared to a control, optionally a previous sample.

49. A method of measuring a level of A-beta in a subject, the method comprising administering to a subject at risk or suspected of having or having AD, an immunoconjugate comprising an antibody of claims 36 or 37 wherein the antibody is conjugated to a detectable label; and detecting the label, optionally quantitatively detecting the label.

50. The method of claim 49, wherein the label is a positron emitting radionuclide.

51. A method of inhibiting A-beta oligomer propagation, the method comprising contacting a cell or tissue expressing A-beta with or administering to a subject in need thereof an effective amount of an A-beta oligomer specific or selective antibody or immunoconjugate of any one of claims 1 to 37, to inhibit A-beta aggregation and/or oligomer propagation.

52. A method of treating AD and/or other A-beta amyloid related diseases, the method comprising administering to a subject in need thereof i) an effective amount of an antibody or immunoconjugate of any one of claims 1-37, or a pharmaceutical composition comprising said antibody; or 2) a nucleic acid or vector comprising a nucleic acid encoding said antibody, to a subject in need thereof.

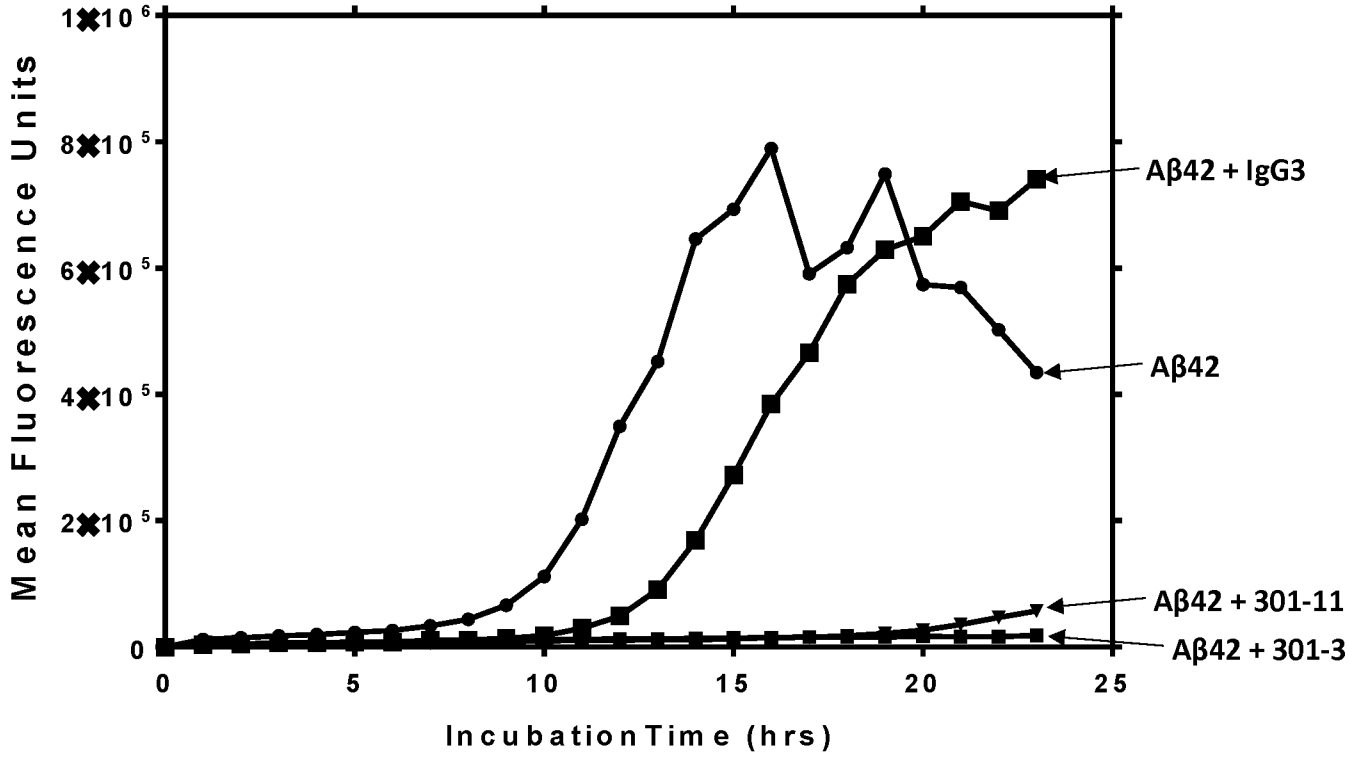
53. The method of claim 52, wherein a biological sample from the subject to be treated is assessed for the presence or levels of A-beta using an antibody described herein.

54. The method of claim any one of claims 51 to 53, wherein the antibody, immunoconjugate, composition or nucleic acid or vector is administered directly to the brain or other portion of the CNS.

55. The method of any one of claims 51 to 54, wherein the composition is a pharmaceutical composition comprising the antibody or immunoconjugate in admixture with a pharmaceutically acceptable, diluent or carrier.

56. A hybridoma cell or cell line expressing the antibody of any one of claims 1 to 16 or expressing an antibody that competes with an antibody comprising or consisting of CDRs selected from the CDRS in Table 2.

Figure 1


Mean Fluorescence Units
1✖10 6
8✖10 5
6✖10 5
4✖10 5
2✖10 5
0
0
5
10
15
20
25
Incubation Time (hrs)
Aβ42 + IgG3
Aβ42
Aβ42 + 301-11
Aβ42 + 301-3

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2017/050866

A. CLASSIFICATION OF SUBJECT MATTER
IPC: ***C07K 16/18*** (2006.01) , ***A61K 47/68*** (2017.01) , ***A61K 49/00*** (2006.01) , ***A61K 51/10*** (2006.01) , ***C07K 16/46*** (2006.01) , ***C12N 15/13*** (2006.01) (more IPCs on the last page)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
Keywords used across the whole IPC

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)
Canadian Patents Database, Questel-Orbit, PubMed, Google Scholar, GQ-Pat, PDP-Protein, Genpept, ENSEMBL, Swiss-Prot, RefSeq, EMBL
Keywords: amyloid, oligomer, antibody, epitope, cyclic peptide, CGHHQKG, HHQK, SEQ ID NOs: 1-6 and 74-83
Authors: Cashman, N.R. and Plotkin, S.S.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WANG, J. et al. "Effects of an amyloid-beta 1-42 oligomers antibody screened from a phage display library in APP/PS1 transgenic mice". Brain Res. 15 March 2016 (15-03-2016), vol. 1635, pp. 169-179, ISSN 0006-8993.	1-56

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:
"A"	document defining the general state of the art which is not considered to be of particular relevance
"E"	earlier application or patent but published on or after the international filing date
"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)
"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
"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
"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
"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
"&"	document member of the same patent family

Date of the actual completion of the international search 28 August 2017 (28-08-2017)	Date of mailing of the international search report 14 September 2017 (14-09-2017)
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.: 819-953-2476	Authorized officer Ernest Tan (819) 639-7658

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA2017/050866

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.: 49-55
because they relate to subject matter not required to be searched by this Authority, namely:

Claims 49-55 are directed to a method of medical treatment of the human or animal body by surgery or therapy, which the International Searching Authority is not required to search in view of PCT Rule 39.1(iv). However, this Authority has nevertheless carried out a search based on the alleged effect or purpose/use of the product defined therein. Although claims 49-50 are directed to a method of measuring a level of A-beta in a subject, the method involves administering an antibody to the subject, which could result in a therapeutic benefit.

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.

CONTINUATION OF SECOND SHEET, BOX A:

C12N 5/10 (2006.01) , ***C12N 5/16*** (2006.01) , ***C12P 21/08*** (2006.01) , ***G01N 33/53*** (2006.01)

专利名称(译)	抗淀粉样蛋白抗体		
公开(公告)号	EP3484919A4	公开(公告)日	2020-03-04
申请号	EP2017830149	申请日	2017-07-18
[标]申请(专利权)人(译)	英属哥伦比亚大学 PROMIS NEUROSCI		
申请(专利权)人(译)	加拿大不列颠哥伦比亚大学 PROMIS神经科学INC.		
当前申请(专利权)人(译)	加拿大不列颠哥伦比亚大学 PROMIS神经科学INC.		
[标]发明人	CASHMAN NEIL R PLOTKIN STEVEN S SILVERMAN JUDITH MAXWELL GIBBS EBRIMA		
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IPC分类号	C07K16/18 A61K47/68 A61K49/00 A61K51/10 C07K16/46 C12N15/13 C12N5/10 C12N5/16 C12P21/08 G01N33/53		
CPC分类号	A61K51/1018 A61K2039/505 A61P25/28 C07K16/18 C07K2317/24 C07K2317/33 C07K2317/34 C07K2317/76 C07K2317/92 G01N2333/4709 C12N5/16		
优先权	62/363566 2016-07-18 US PCT/CA2016/051303 2016-11-09 WO 62/443766 2017-01-08 US 62/507587 2017-05-17 US 62/507633 2017-05-17 US		
其他公开文献	EP3484919A1		
外部链接	Espacenet		

摘要(译)

本公开内容涉及结合A-β寡聚体的抗体以及制备和使用所述抗体的方法。