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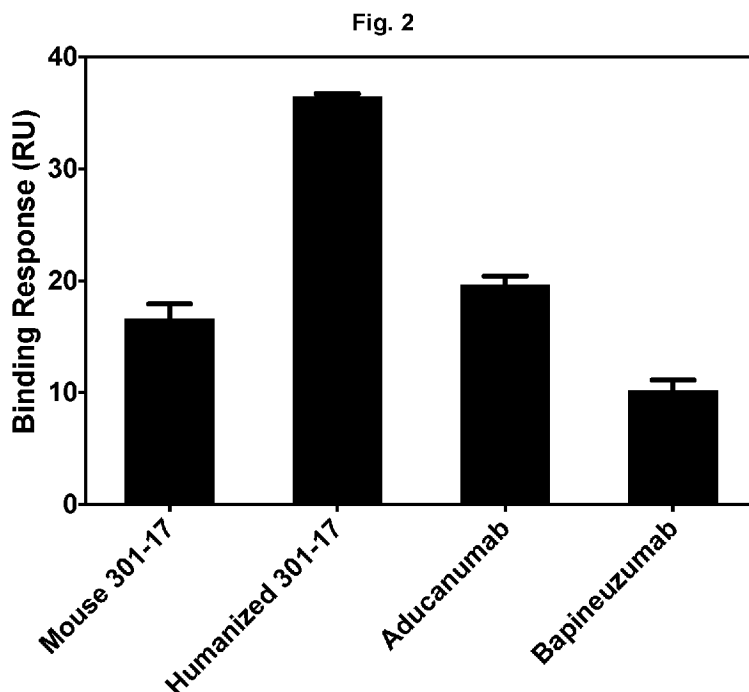
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(54) Title: ANTIBODIES TO AMYLOID BETA



(57) Abstract: The disclosure pertains to antibodies that bind A-beta oligomers and methods of making and using said antibodies. Also provided are chimeric or humanized antibodies, including antibodies having CDRs in Table 2 and/or having a sequence as set forth in Table 4B or a sequence with at least 50% sequence identity thereto optionally wherein the CDR amino acid sequences are as set forth in SEQ ID Nos: 74-79. Also provided are methods and uses thereof as well as kits comprising said antibodies.



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5 **Title: Antibodies to Amyloid beta**

**Related Applications**

This PCT application claims the benefit of priority to PCT Application PCT/CA2017/050866 filed July 18, 2017 and US provisional application US 62/622, 126 filed January 25, 2018, each of which are  
10 incorporated in their entirety.

**Field**

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

**Sequence Listing**

15 **[0002]** A computer readable form of the Sequence Listing "P50442PC02SL\_asfiled.txt" (82,028 bytes), submitted via EFS-WEB and created on July 18, 2018, is herein incorporated by reference.

**Background**

20 **[0003]** 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  $\beta$  and  $\gamma$  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.

25 **[0004]** 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 $\beta$  structure (PDB entry, 2M4J); a two-fold symmetric structure of A $\beta$ -40 monomers (PDB entry 2LMN), and a single-chain, parallel in-register structure of A $\beta$ -42 monomers (PDB entry 2MXU).

30 **[0005]** 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].

**[0006]** 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.

35 **[0007]** 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.

**[0008]** Antibodies that bind A-beta have been described.

5 [0009] 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.

[0010] 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.

10 [0011] 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.

15 [0012] US20110171243A1 titled COMPOUNDS TREATING AMYLOIDOSES discloses a peptide mimotope capable of inducing the *in vivo* formation of antibodies that bind HQKLVF and/or HQKLVFFAED, and its use.

[0013] 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].

20 [0014] 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.

[0015] 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).

30 [0016] 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 A $\beta$  peptide. WO 01/62801 describes antibodies that bind A-beta between amino acids 13-28. WO2004071408 discloses humanized antibodies.

35 [0017] 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.

40 [0018] WO2003070760 titled ANTI-AMYLOID BETA ANTIBODIES AND THEIR USE is directed towards antibody molecules capable of specifically recognizing two regions of the R-A4

5 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.

[0019] WO2006066089 titled HUMANIZED AMYLOID BETA ANTIBODIES FOR USE IN IMPROVING COGNITION is directed to improved agents and methods for treatment of diseases  
10 associated with beta amyloid and in particular to the identification and characterization of a monoclonal antibody, 12A11, that specifically binds to A $\beta$  peptide and is effective at reducing plaque burden associated with amyloidogenic disorders (e.g., AD).

[0020] WO2007068429 titled ANTIBODIES AGAINST AMYLOID BETA 4 WITH GLYCOSYLATED IN THE VARIABLE REGION is directed to a purified antibody molecule preparation  
15 being characterized in that at least one antigen binding site comprises a glycosylated asparagine (Asn) in the variable region of the heavy chain (V<sub>H</sub>).

[0021] 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  
20 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.

[0022] Yu et al. describes a hexavalent foldable A $\beta$ 1-15 (6A $\beta$ 15) 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  
25 Disease [14], [15].

[0023] Antibodies that preferentially or selectively bind A-beta oligomers over monomers or over fibrils or over both monomers and fibrils are desirable.

### Summary

[0024] An aspect includes an isolated humanized antibody having CDRs comprising or  
30 consisting of SEQ ID NOs: 74-79 or SEQ ID Nos:1-6 or SEQ ID Nos:1, 2, 80 and 4-6 or SEQ ID Nos: 1, 2, 80-83 and having 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 or 4B or having at least 50% sequence identity to a sequence selected from the sequences in Table 4A or 4B, wherein the CDR amino acid sequences are as shown therein or selected from SEQ ID NOs: 74-79 or SEQ ID Nos:1-6  
35 or SEQ ID Nos:1, 2, 80 and 4-6 or SEQ ID Nos: 1, 2, 80-83.

[0025] 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.

[0026] In another embodiment, the antibody is an antibody binding fragment of an antibody  
40 described herein selected from Fab, Fab', F(ab')<sub>2</sub>, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies, diabodies, and multimers thereof.

- 5 [0027] An aspect includes immunoconjugate comprising the humanized antibody described herein and a detectable label or cytotoxic agent.
- [0028] In an embodiment, the detectable label comprises a positron emitting radionuclide, optionally for use in subject imaging such as PET imaging.
- [0029] An aspect includes a composition comprising the antibody described herein, or the  
10 immunoconjugate described herein, optionally with a diluent.
- [0030] 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.
- [0031] An aspect includes a vector comprising the nucleic acid described herein.
- 15 [0032] An aspect includes a cell expressing an antibody described herein, optionally wherein the cell is a hybridoma comprising the vector described herein.
- [0033] 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.
- 20 [0034] An aspect includes a method of determining if a biological sample comprises A-beta, the method comprising:
- a. contacting the biological sample with a humanized antibody described herein or the immunoconjugate described herein; and
  - b. detecting the presence of any antibody complex.
- 25 [0035] In an embodiment, the biological sample contains A-beta oligomer the method comprising:
- a. contacting the sample with a humanized 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
  - 30 b. detecting the presence of any complex;
- wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.
- [0036] In another embodiment, the amount of complex is measured.
- [0037] In another embodiment, the sample comprises brain tissue or an extract thereof, whole  
35 blood, plasma, serum and/or CSF.
- [0038] In another embodiment, the sample is a human sample.
- [0039] In another embodiment, the sample is compared to a control, optionally a previous sample.

5 [0040] 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.

[0041] 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 a humanized antibody described herein wherein the humanized antibody is conjugated to a detectable label; and detecting the label, optionally quantitatively detecting the label.  
10

[0042] In an embodiment, the label is a positron emitting radionuclide.

[0043] 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.  
15

[0044] 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 a chimeric antibody, humanized 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.  
20

[0045] 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.

[0046] In another embodiment, the antibody, immunoconjugate, composition or nucleic acid or vector is administered directly to the brain or other portion of the CNS.  
25

[0047] 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.  
30

#### **Brief description of the drawings**

[0048] An embodiment of the present disclosure will now be described in relation to the drawings in which:

[0049] FIG. 1 is a graph reporting effect of antibodies on propagation of A-beta oligomers in vitro.  
35

[0050] FIG. 2 is a graph showing that the humanized antibody shows significantly better binding to the toxic oligomer enriched LMW fraction of AD extract.

[0051] FIGs. 3A to 3F are a series of brain sections stained with various Abeta and control antibodies.

- 5 [0052] FIG 4A is a trace an AD soluble brain extract separated
- [0053] FIGs. 4B to 4D are graphs showing the levels of A-beta detected in LMW and HMW fractions.
- [0054] FIG. 5A is a graph showing levels of human IgG detectable in brain and plasma at 24hrs after injection of anti-Abeta antibodies.
- 10 [0055] FIG. 5B is a graph showing levels of anti-Abeta antibody CNS penetrance.
- [0056] FIGs. 5C and 5D show levels of IgG detected in brain and plasma or non-transgenic and App/PS1 mice 1-21 days after injection of anti-Abeta antibodies.

#### **Detailed description of the Disclosure**

15 [0057] Provided herein are chimeric and humanized antibodies comprising CDRs having sequences as shown in Table 2, and/or having variable region sequences provided in any of Tables 4A and 4B 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).

20 [0058] 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  
25 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 A $\beta$  oligomer propagation and aggregation.

[0059] As further shown in the Examples, humanized antibody 301-17 showed improved characteristics such as improved binding of oligomeric A-beta compared to the mouse monoclonal  
30 antibody. The humanized antibodies also had improved specificity compared to the parental monoclonal.

[0060] Accordingly in some embodiments the humanized antibody has increased binding, such as 50% increased or up to 200% increased (or any number inbetween) compared to mouse monoclonal antibody, to oligomeric A-beta, optionally oligomeric Abeta present in low molecular weight  
35 fractions of samples such as brain extracts or other biological samples.

[0061]

#### **I. Definitions**

[0062] As used herein, the term 'A-beta' may alternately be referred to as 'amyloid beta', 'amyloid  $\beta$ ', A-beta, A-beta or 'A $\beta$ '. Amyloid beta is a peptide of 36-43 amino acids and includes all wild-  
40 type and mutant forms of all species, particularly human A-beta. A-beta40 refers to the 40 amino acid

5 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.

**[0063]** 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.

10 **[0064]** 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  
15 in the brain and CSF of a subject with AD.

**[0065]** 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  
20 of monomers. Fibrils can aggregate by the thousands to form senile plaques, one of the primary pathological morphologies diagnostic of AD.

**[0066]** 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  
25 compound.

**[0067]** 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.

30 **[0068]** 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  
35 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.

**[0069]** 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  
40 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.

5 **[0070]** 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')<sub>2</sub>, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies,  
diabodies, and multimers thereof. Fragments can be obtained via chemical or enzymatic treatment of  
10 an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant  
means. For example, F(ab')<sub>2</sub> fragments can be generated by treating the antibody with pepsin. The  
resulting F(ab')<sub>2</sub> 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')<sub>2</sub>, scFv, dsFv, ds-scFv,  
dimers, minibodies, diabodies, bispecific antibody fragments and other fragments can also be  
15 constructed by recombinant expression techniques.

**[0071]** 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.

20 **[0072]** 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  
25 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  
30 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  
35 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.

**[0073]** 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  
40 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.

5 **[0074]** 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.

10 **[0075]** 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  $^3\text{H}$ ,  $^{13}\text{N}$ ,  $^{14}\text{C}$ ,  $^{18}\text{F}$ ,  $^{32}\text{P}$ ,  $^{35}\text{S}$ ,  
15  $^{123}\text{I}$ ,  $^{125}\text{I}$ ,  $^{131}\text{I}$ ; 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.

**[0076]** The term "epitope" as commonly used means an antibody binding site, typically a  
20 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  
25 present specification when it is accessible to binding by an antibody.

**[0077]** 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 ( $K_{\text{on}}$ ) and/or with a smaller dissociation constant ( $K_{\text{off}}$ ) 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  
30 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  $k_{\text{on}}/k_{\text{off}}$ . The  $k_{\text{on}}$  and  $k_{\text{off}}$  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  
35 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).

**[0078]** 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  
40 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.

5 **[0079]** 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  
 10 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.

**[0080]** 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  
 15 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.

**[0081]** The term "animal" or "subject" as used herein includes all members of the animal kingdom including mammals, optionally including or excluding humans.

20 **[0082]** 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	
<u>Type of Amino Acid</u>	<u>Substitutable Amino Acids</u>
Hydrophilic	Ala, Pro, Gly, Glu, Asp, Gln, Asn, Ser, Thr
Sulphydryl	Cys
Aliphatic	Val, Ile, Leu, Met
Basic	Lys, Arg, His
Aromatic	Phe, Tyr, Trp

25  
 30 **[0083]** 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  
 35 positions shared by the sequences (i.e., % identity=number of identical overlapping positions/total

5 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.

10 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

15 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

20 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

25 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.

**[0084]** 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

30 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.

35 **[0085]** 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

40 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

5 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  
10 located at the 5' and 3' ends of the nucleic acid) from which the nucleic acid is derived.

**[0086]** "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  
15 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  
20 expression vector.

**[0087]** 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  
25 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.

**[0088]** 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  
30 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  $T_m$ ,  
which in sodium containing buffers is a function of the sodium ion concentration and temperature ( $T_m$   
 $= 81.5^\circ\text{C} - 16.6 (\text{Log}_{10} [\text{Na}^+]) + 0.41(\%(\text{G}+\text{C}) - 600/\text{l})$ , or similar equation). Accordingly, the parameters  
in the wash conditions that determine hybrid stability are sodium ion concentration and temperature. In  
35 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  $1^\circ\text{C}$  decrease in  $T_m$ , for example if nucleic acid  
molecules are sought that have a >95% identity, the final wash temperature will be reduced by about  
 $5^\circ\text{C}$ . 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  
40 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  $T_m - 5^\circ\text{C}$   
based on the above equation, followed by a wash of 0.2x SSC/0.1% SDS at  $60^\circ\text{C}$ . Moderately stringent  
hybridization conditions include a washing step in 3x SSC at  $42^\circ\text{C}$ . It is understood, however, that

5 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.

10 **[0089]** 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  
15 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.

20 **[0090]** 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.

**[0091]** 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.  
25 Dosage regime may be adjusted to provide the optimum therapeutic response.

**[0092]** 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.

30 **[0093]** 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.

**[0094]** 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  
35 unstated features, elements, components, groups, integers and/or steps.

**[0095]** 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  
40 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.

5 **[0096]** 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.

**[0097]** 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.

## 15 **II. Antibodies and Nucleic acids**

**[0098]** Disclosed herein are particular antibodies and uses thereof.

**[0099]** 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.

**[00100]** 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 of SEQ ID NOs: 1-3 and 4-6.

**[00101]** 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.

**[00102]** 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.

**[00103]** 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.

- 5 **[00104]** 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.
- [00105]** 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.
- 10 **[00106]** 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.
- [00107]** 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 SEQ ID NOS 1, 2, 80 and 4-6.
- 15 **[00108]** 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.
- 20 **[00109]** 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.
- 25 **[00110]** 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.
- 30 **[00111]** 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.
- [00112]** 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.
- 35 **[00111]** 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.
- 40 **[00112]** 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.

5 **[00113]** 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.

10 **[00114]** 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 SEQ ID NOs: 1, 2, 80 and 81-83.

15 **[00115]** 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  
20 and 80.

**[00116]** 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  
25 amino acid sequence of i) wherein the CDR sequences are as set forth in SEQ ID NO: 81, 82 and 83.

**[00117]** 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  
30 version thereof.

**[00118]** 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.

35 **[00119]** 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.

**[00120]** 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  
40 SEQ ID NO: 89.

5 **[00121]** 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.

**[00122]** 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.

10 **[00123]** 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.

**[00124]** 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.

**[00125]** 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).

**[00126]** In an embodiment, the antibody is a monoclonal antibody. The production of monoclonals is described in the Examples.

**[00127]** 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.

5 [00128] 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);  
10 and McCafferty et al., Nature 348:552-554 (1990).

[00129] In an embodiment, the antibody is a chimeric antibody, optionally comprising CDRs in Table 2, and an IgG4 framework, optionally comprising the heavy chain variable region of SEQ ID NO 42, 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: 42, wherein the CDR sequences are SEQ ID NO: 74-76, or a  
15 conservatively substituted amino acid sequence of SEQ ID NO: 42, wherein the CDR sequences are the sequences SEQ ID NO: 74-76.

[00130] In an embodiment, the antibody is a chimeric antibody, optionally comprising CDRs in Table 2, and an IgG4 framework, optionally comprising the light chain variable region of SEQ ID NO 56, an amino acid sequence with at least 50%, at least 60%, at least 70%, at least 80%, or at least  
20 90% sequence identity to SEQ ID NO: 56, wherein the CDR sequences are SEQ ID NO: 77-79, or a conservatively substituted amino acid sequence of SEQ ID NO: 56, wherein the CDR sequences are the sequences SEQ ID NO: 77-79.

[00131] In an embodiment, the antibody is a humanized antibody. As demonstrated in the Examples, specific humanized antibodies are described.

25 [00132] The humanization of antibodies from non-human species has been 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.).

[00133] Humanized forms of rodent antibodies can be 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  
30 or other recombinant techniques and may be aided by computer modeling of the antigen binding site  
35 (Co et al. J Immunol, 152: 2968-2976, 1994).

[00134] The method described in the Examples can also be used.

[00135] The method can for example the variable region can be grafted into a human framework and for example the introduction of specific mutations can be introduced into the variable  
40 region outside of the CDRs.

- 5 **[00136]** 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.
- [00137]** In an embodiment, the humanized antibody comprises CDRs as shown in Table 2.
- [00138]** Specific humanized sequences are provided in Tables 4A and 4B.
- 10 **[00139]** 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.
- [00140]** 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;
- 15 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.
- [00141]** In another embodiment, the antibody comprises a light chain variable region comprising
- 20 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.
- 25 **[00142]** 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.
- 30 **[00143]** 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.
- [00144]** In another embodiment, the antibody is an antibody that competes for binding to a
- 35 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.
- [00145]** In another embodiment, the antibody is an antibody that competes for binding to a
- 40 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

5 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.

**[00146]** 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  
10 90% sequence identity thereto wherein the CDRs are maintained as shown in Table 2.

**[00147]** In another embodiment, the humanized antibody comprises a sequence as shown in Table 4B.

**[00148]** 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;  
15 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).

20 **[00149]** 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  
25 amino acid sequence of i) wherein the CDR sequences are the sequences shown underlined therein (also in SEQ ID NOs:77-79).

**[00150]** 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  
30 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.

**[00151]** 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  
35 of SEQ ID NO: 58, 60, 62, 64, 66 and 68.

**[00152]** 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.

40 **[00153]** 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

5 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.

**[00154]** 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  
10 90% sequence identity thereto wherein the CDRs are maintained as shown underlined therein (also in in SEQ ID Nos:74-79).

**[00155]** 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  
15 SEQ ID NO:70 and/or 72; or iii) a conservatively substituted amino acid sequence i).

**[00156]** 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  
20 version thereof.

**[00157]** In an embodiment, the humanized antibody comprises the sequences of VH2 Vk5.

**[00158]** In an embodiment, the humanized antibody is a Fab fragment.

**[00159]** In an embodiment, the Fab fragment comprises any of the variable regions in Table 4A and 4B. in an embodiment the Fab fragment comprises VH2 Vk5.

25 **[00160]** In a further embodiment, the antibody comprises the CH1 and/or CL portion of IgG4 in Table 5, a conservative variant thereof or a sequence with at least 50%, 60%, 70%, 89%, 90% or 95% sequence identity thereto. In a further embodiment, the antibody comprises sequences in Table 5 or a conservative variant thereof or a sequence with at least 50%, 60%, 70%, 89%, 90% or 95% sequence identity thereto.

30 **[00161]** In an embodiment, the antibody comprises a KD of at least or about  $1 \times 10^{-10}$ , at least or about  $8 \times 10^{-11}$ , at least or about  $6 \times 10^{-11}$ , at least or about  $4 \times 10^{-11}$  or at least or about  $2 \times 10^{-11}$  for the cyclic peptide with sequence of SEQ ID NO: 12.

**[00162]** 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  
35 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  
40 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

5 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.

**[00163]** 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  
10 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.

**[00164]** Humanized antibodies are typically produced as antigen binding fragments such as Fab, Fab' F(ab')<sub>2</sub>, Fd, Fv and single domain antibody fragments, or as single chain antibodies in which the  
15 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).

**[00165]** 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  
20 IgG4. The humanized or human antibody may include sequences from one or more than one isotype or class.

**[00166]** 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.

**[00167]** In an embodiment, the antibody has a sequence or a part thereof as provided in Table  
25 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.

**[00168]** 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  
30 sequences of antibodies with CDRS having SEQ ID NOs:1-6, or 1, 2, 80, 4-6 or 1, 2, 80-83.

**[00169]** 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  
35 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.

**[00170]** 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  
40 recited in Table 2.

5 **[00171]** 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.

**[00172]** The antibodies herein can be single chain antibodies. The humanized antibodies described are also in an embodiment, single chain antibodies.

10 **[00173]** 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.

**[00174]** Competition between antibodies can be determined for example using an assay in  
15 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.

20 **[00175]** In an embodiment the antibody is isolated. In an embodiment, the antibody is an exogenous antibody.

**[00176]** 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.

25 **[00177]** In another embodiment, the antibody does not selectively bind monomeric A-beta compared to native- or synthetic- oligomeric A-beta.

**[00178]** In an embodiment, the A-beta oligomer comprises A-beta 1-42 subunits.

**[00179]** In an embodiment, the antibody lacks A-beta fibril plaque (also referred to as senile  
30 plaque) staining, for example as measured by immunohistochemistry. 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  
35 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.

**[00180]** 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  
40 radionuclide can be used for example in PET imaging.

5 **[00181]** A further aspect relates to an antibody complex comprising an antibody described herein and/or a binding fragment thereof and oligomeric A-beta.

**[00182]** A further aspect is an isolated nucleic acid encoding an antibody or part thereof described herein.

10 **[00183]** 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.

15 **[00184]** 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  
20 sequences encoding any of the amino acid sequences shown in Tables 2, 3, 4A, 4B and 8.

**[00185]** 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.

**[00186]** Another aspect is an expression cassette or vector comprising the nucleic acid herein disclosed. In an embodiment, the vector is an isolated vector.

25 **[00187]** 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.

**[00188]** 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.

30 **[00189]** 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.

40 **[00190]** Suitable regulatory sequences may be derived from a variety of sources, including bacterial, fungal, viral, mammalian, or insect genes.

- 5 **[00191]** 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.
- 10 **[00192]** In an embodiment, the regulatory sequences direct or increase expression in neural tissue and/or cells.
- [00193]** In an embodiment, the vector is a viral vector.
- [00194]** 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.
- 15 **[00195]** 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.
- 20 **[00196]** 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).
- 25 **[00197]** 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.
- 30 **[00198]** 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.
- 35 **[00199]** 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
- 40

5 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.

**[00200]** 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.

10 **[00201]** In an embodiment, the cell is a eukaryotic cell selected from a yeast, plant, worm, insect, avian, fish, reptile and mammalian cell.

**[00202]** In another embodiment, the mammalian cell is a myeloma cell, a spleen cell, or a hybridoma cell.

**[00203]** In an embodiment, the cell is a neural cell.

15 **[00204]** 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. cerevisiae* 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  
20 art.

**[00205]** 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.

25 **[00206]** 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.

30 **[00207]** A further aspect is a hybridoma cell line producing an antibody comprising the a CDR set described herein.

### **III. Compositions**

35 **[00208]** A further aspect is a composition comprising a nucleic acid, vector or antibody described herein.

**[00209]** In an embodiment, the composition comprises a diluent.

**[00210]** Suitable diluents for nucleic acids include but are not limited to water, saline solutions and ethanol.

5 [00211] 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.

[00212] 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.

10 [00213] 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.

15 [00214] 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.

[00215] 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-dioleoyloxy)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.

[00216] 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-ethylamino ethanol,

35 [00217] 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.

- 5 [00218] 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**

- 10 [00219] 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.

[00220] In an embodiment, the kit further comprises one or more of a collection vial, standard buffer and detection reagent.

- 15 [00221] In another embodiment, the kit is for diagnosing or monitoring Alzheimer's disease or a condition involving oligomeric Abeta.

#### **V. Methods**

[00222] Included are methods for making the antibodies described herein.

- 20 [00223] 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.

- 25 [00224] In an embodiment, the method is for making a monoclonal antibody using for example a method as described herein.

- [00225] 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.
- 30

- [00226] 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.
- 35

- [00227] 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
- 40

5 bind oligomer and/or lack or negligibly bind plaque in situ and/or corresponding linear peptide, optionally using a method described herein.

**[00228]** 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  
10 whether a biological sample comprises oligomeric A-beta.

**[00229]** 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

15 b. detecting the presence of any complex;  
wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.

**[00230]** In an embodiment, the level of complex formed is compared to a test antibody such as a suitable Ig control or irrelevant antibody.

**[00231]** In an embodiment, the detection is quantitated and the amount of complex produced is  
20 measured. The measurement can for example be relative to a standard.

**[00232]** In an embodiment, the measured amount is compared to a control.

**[00233]** 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;

25 (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.

**[00234]** The control can be a sample control (e.g. from a subject without AD, or from a subject  
30 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.

**[00235]** 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  
35 chimeric antibody.

**[00236]** 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.

5 **[00237]** 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.

10 **[00238]** 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.

15 **[00239]** 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  
20 can for example be used in PET imaging.

**[00240]** 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  
25 synaptic metabolism.

**[00241]** Detecting A-beta levels using an antibody described herein can be used alone or in combination with other methods to monitor response to treatment.

**[00242]** 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.  
30 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  
35 antibody described herein to inhibit A-beta aggregation and/or oligomer propagation. In vitro the assay can be monitored as described in the Examples.

**[00243]** 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 inclusion body myositis (a muscle disease) exhibit similar plaques as AD and A-beta can also form aggregates implicated in cerebral  
40 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.

5 [00244] 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  
10 described herein can also be administered to the subject, optionally using vectors suitable for delivering  
nucleic acids in a subject.

[00245] 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)  
15 is treated with the antibody.

[00246] 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.

[00247] One or more antibodies targeting HHQK (SEQ ID NO: 7) can be administered in  
20 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.

[00248] Also provided are uses of the compositions, antibodies, isolated peptides, and nucleic  
acids for treating AD or A-beta amyloid related diseases.

[00249] The compositions, antibodies, isolated peptides and nucleic acids, vectors etc.  
25 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.

[00250] In certain embodiments, the pharmaceutical composition is administered systemically.

[00251] In other embodiments, the pharmaceutical composition is administered directly to the  
30 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.  
35 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).

5 [00252] 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.

[00253] Other embodiments contemplate the co-administration of the compositions, antibodies, isolated peptides and nucleic acids described herein with biologically active molecules known to  
10 facilitate the transport across the blood brain barrier.

[00254] 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",  
15 herein incorporated by reference.

[00255] 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  
20 render expedient. Although specific terms have been employed herein, such terms are intended in a descriptive sense and not for purposes of limitation.

[00256] The following non-limiting examples are illustrative of the present disclosure:

### Examples

#### 25 Example 1

##### Antibody Generation

##### Methods and Materials

##### Immunogen

[00257] Cyclo(CGHHQKG) (SEQ ID NO:12) peptide was generated at CPC Scientific,  
30 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.

##### Antibodies

[00258] Hybridomas and monoclonal antibodies were generated to cyclo(CGHHQKG) (SEQ  
35 ID NO: 12) linked to Keyhole Limpet Hemocyanin (KLH).

##### Fusion / Hybridoma Development

[00259] 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. T.

##### Hybridoma Analysis

40 [00260] 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. Positive cultures were retested on

5 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.

10

#### ELISA Antibody Screening

**[00261]** 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.

20

#### SPR Binding Assays

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

##### **A-beta Monomer and Oligomer Preparation:**

**[00262]** 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 dH<sub>2</sub>O 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.

##### **SPR Analysis of Cyclic Peptide, A-beta Monomer and Oligomer binding:**

**[00263]** 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.

**[00264]** 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

5 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.

## 10 **Antibody Sequencing**

**[00265]** 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.

15

## **Humanized Antibodies**

**[00266]** Humanized IgG4 antibody constructs were prepared for 301-17 and sequenced (Abzena Cambridge UK).

**[00267]** Briefly RNA was extracted from the hybridoma 301-17 cell pellet using an RNeasy Mini Kit (Qiagen, Hilden, Germany). V-regions were amplified by RT-PCR using degenerate primer pools for murine antibody signal sequences together with constant region primers for each of IgG and Igk. Heavy chain V region mRNA was amplified using a set of six degenerate primer pools (A to F) specific for VH signal sequences together with IgG-specific constant region primers. The light chain V region mRNA was amplified using a set of eight signal sequence-specific degenerate primer pools, seven for the  
20 kappa cluster (Igk-A to Igk-G) and one for the lambda cluster (Igλ), together with κ or λ constant region primers. The PCR products obtained were purified, cloned into a 'TA' cloning vector (pGEM-T Easy, Promega, Madison, USA), transformed into *E. coli* and individual colonies sequenced.

**[00268]** Chimeric constructs (V0H0 and V0k0) were prepared using the variable regions from the hybridoma which were cloned into a human IgG4 framework. The chimeric constructs were then  
30 humanized to create 6 humanized heavy chains (VH1-6) and 6 light chains (Vk1-6). VH1-6 and Vk4-6 constructs were mixed to create different humanized antibodies e.g. VH2Vk4.

**[00269]** The S241P hinge variant comprises an altered disulfide bond arrangement of an IgG4 molecule by mutation of the Cys at the N terminus of the heavy chain constant domain 1 (C<sub>H</sub>1) (Kabat position 127) to a Ser and introduction of a Cys at a variety of positions (positions 227–230) at the C  
35 terminus of C<sub>H</sub>1. An inter-LC-C<sub>H</sub>1 disulfide bond is formed [17].

**[00270]** The fully humanized antibodies were prepared using Composite Human Antibody™ technology. The humanized variable region genes were cloned into vectors encoding a human IgG4 (S241P hinge variant) heavy chain constant domain and a human kappa light chain constant domain. Chimeric and humanized antibodies were transiently expressed in CHO cells and Protein A purified and  
40 tested. All 301-17 humanized antibodies selectively bound the cyclic peptide conjugated to BSA with binding affinities within 2-fold of the reference chimeric antibody and greater than 10 fold and about 20 of the reference monoclonal antibody. The chimeric and humanized antibodies had a KD(M) of about 2

5 x 10<sup>-11</sup> whereas the KD of the monoclonal antibody had a KD (M) of 4 x 10<sup>-10</sup> for the cyclic peptide. As shown in Fig. 2 this translates to an improved affinity for oligomeric Abeta.

[00271] Binding was determined using single cycle Biacore analysis. Antibodies were analysed in two separate experiments.

10 [00272] Humanized antibody sequences are provided in Table 4B (301-17). The CDR sequences of each antibody sequences are bolded and underlined. The CDRs of 301-11 or any other antibody described herein can be used to replace the CDRs in the humanized constructs as shown for example in Table 4A.

**Results**

15 [00273] 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.

[00274] Isotyping revealed 301-3, 301-11 and 301-17 were IgG3 subtypes.

[00275] 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.

20 [00276] ELISA and SPR assays confirmed that the clones preferentially bound the cyclopeptide 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.

25 [00277] 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**

	<b>Cyclic Peptide(RU)</b>	<b>Linear Peptide(RU)</b>	<b>Ab42 Monomer(RU)</b>	<b>Ab42 Oligomer(RU)</b>
<b>301-11</b>	<b>488</b>	<b>210.5</b>	<b>21.6</b>	<b>75.3</b>
<b>301-3</b>	<b>468.9</b>	<b>60.6</b>	<b>-1.8</b>	<b>56.8</b>

**Table 1B**

	<b>Ab42 Monomer (RU)</b>	<b>Ab42 Oligomer(RU)</b>
<b>301-3</b>	<b>-23.8</b>	<b>15.5</b>
<b>301-11</b>	<b>-14.1</b>	<b>-2.8</b>
<b>301-17</b>	<b>-27.1</b>	<b>147.8</b>

**Antibody Sequence**

30 [00278] 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

- 5 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.

[00279] 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.

- [00280] Two light chains were sequenced. One light chain was near identical to the  
10 light chain for 301-11.

[00281] 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.

15 **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	QSLNLSRTRKNY	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	ARDYYGSNSYTS GFAY	80
301-03-1	Light	CDR-L1	QSLNLSRTRKNY	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	ARDYYGSNSYTS GFAY	80
301-03-2	Light	CDR-L1	QSI VHSNGNTY	81
301-03-2		CDR-L2	KVS	82
301-03-2		CDR-L3	FQGSHPVPLT	83
301-17	Heavy	<u>CDR-H1</u>	GYSFTSYW	74
301-17		CDR-H2	VHPGRGVST	75
301-17		CDR-H3	SRSHGNTYWF FDV	76
301-17	Light	CDR-L1	QSI VHSNGNTY	77
301-17		CDR-L2	KVS	78
301-17		CDR-L3	FQGSHPVFT	79

**Table 3**

- 20 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	ATGAAC TTTGGGCTCAGCTTGATTTTCCTTGCTCTGTTTTAAAA GGTGTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA GTGAAGCCTGGAGGGTCCCTGAAACTCTCTGTGCAGCCTCT <b><u>GGA</u></b> <b><u>TTCACTTT CAGT GACT ATTAC</u></b> ATGTATTGGGTTCGCCAGACTC	MNFGLSLIFLVLVLKGVQCEVQLVESGGGLVK PGGSLKLSCAAS <b><u>GFTF</u></b> <b><u>SDYY</u></b> MYWVRQTPEKRL

<p>SEQ ID NO: 8, 9</p>	<p>CGGAAAAGAGGCTGGAGTGGGTGCGCAACC<b><u>ATTAGTGATGGTGG</u></b> <b><u>TAGTTACACC</u></b>TCCATCCAGACAGTGTGAAGGGACGATTCACCA TCTCCAGAGACAATGCCAAGAACAACCTGTACCTGCAAATGAGCA GTCTGAGGCTGAGGACACAGCCATGTATTACTGT<b><u>GCAAGAGAT</u></b> <b><u>TACTACGGTAGTAGTACACCTCGGGCTTTGCTTAC</u></b>TG GGGCCAAGGGACTCTGGTCACTGTCTCTGCA</p>	<p>EWVAT<b><u>ISDGGSYT</u></b>SY PDSVKGRFTISRDNK NNLYLQMSLRSEDTA MYYC<b><u>ARDYYGSSSYT</u></b> <b><u>SGFAY</u></b>WGQGLTVVSA</p>
<p>301-11 Kappa SEQ ID NO: 10,11</p>	<p>ATGGATTACAGGCCAGGTTCTTATATTGCTGCTGCTATGGGTA TCTGGTACCTGTGGGACATTGTGATGTCACAGTCTCCATCCTCC CTGGCTGTGTCAACAGGAGAGAAGGTCATATGAGCTGCAAATCC AGT<b><u>CAGAGTCTGCTCAACAGTAGAACCCGAAAGAACTAC</u></b>TT GGCTTGGTACCAGCAGAAACCAGGGCAGTCTCTAAACTGCTGAT CTAC<b><u>TGGGCATCC</u></b>ACTAGGGAATCTGGGGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTG TGCAGGCTGAAGACCTGGCAGTTTATTACTGC<b><u>AAGCAATCTTAT</u></b> <b><u>AATCTGTACACG</u></b>TTCCGGAGGGGGACCAAGCTGGAAATAAAA</p>	<p>MDSQAQVLIILLWVLS GTCGDIVMSQSPSSLA VSTGEKVTMSCKSS<b><u>QS</u></b> <b><u>LLNSRTRKNY</u></b>LAWYQ QKPGQSPKLLIY<b><u>WAST</u></b> RESGVPDRFTGSGSGT DFTLTISSVQAE DLAV YYC<b><u>KQSYNLYT</u></b>FGGG TKLEIK</p>
<p>301-03 IgG3 SEQ ID NO: 84, 85</p>	<p>ATGAAC TTCGGGCTCAGCTTGATTTTCTTGCTCTGTTTTAAAA GGTGTCCAGTGTGAAGTGCAGCTGGTGGAGTCTGGGGGAGGCTTA GTGAAGCCTGGAGGGTCCCTGAAACTCTCTGTGCAGCCTCT<b><u>GGA</u></b> <b><u>TTCACTTTCAGTGACTATTAC</u></b>ATGTATTGGGTTCGCCAGACTC CGGAAAAGAGGCTGGAGTGGGTGCGCAACC<b><u>ATTAGTGATGGTGG</u></b> <b><u>TAGTTACACC</u></b>TCCATCCAGACAGTGTGAAGGGGCGATTCACCA TCTCCAGAGACAGTGCCAAGAACAACCTGTACCTGCAAATGAGCA GTCTGAAGTCTGAGGACACAGCCATGTATTACTGT<b><u>GCAAGAGAT</u></b> <b><u>TACTACGGTAGTAATAGTTACACCTCGGGCTTTGCTTAC</u></b>TG GGGCCAAGGGACTCTGGTCACTGTCTCTGCA</p>	<p>MNFGLSLIFLVLVLK VQCEVQLVESGGGLVK PGGSLKLSAAS<b><u>GFTF</u></b> <b><u>SDYY</u></b>MYWVRQTPEKRL EWVAT<b><u>ISDGGSYT</u></b>SY PDSVKGRFTISRDSA NNLYLQMSLRKSEDTA MYYC<b><u>ARDYYGSNSYT</u></b> <b><u>SGFAY</u></b>WGQGLTVVSA</p>
<p>301-03 Kappa 1 SEQ ID NO: 86, 87</p>	<p>ATGGATTACAGGCCAGGTTCTTATATTGCTGCTGCTATGGGTA TCTGGTACCTGTGGGACATTGTGATGTCACAGTCTCCATCCTCC CTGGCTGTGTGAGCAGGAGAGAAGGTCATATGAGCTGCAAATCC AGT<b><u>CAGAGTCTGCTCAATAGTAGAACCCGAAAGAACTAC</u></b>TT GGCTTGGTACCAGCAGAAACCAGGGCAGTCTCTAAACTGCTGAT CTAC<b><u>TGGGCATCC</u></b>ACTAGGGAATCTGGGGTCCCTGATCGCTTCA CAGGCAGTGGATCTGGGACAGATTTCACTCTCACCATCAGCAGTG TGCAGGCTGAAGACCTGGCAGTTTATTACTGC<b><u>AAGCAATCTTAT</u></b> <b><u>AATCTGTACACG</u></b>TTCCGGAGGGGGACCAAGCTGGAAATAAAA</p>	<p>MDSQAQVLIILLWVLS GTCGDIVMSQSPSSLA VSAGEKVTMSCKSS<b><u>QS</u></b> <b><u>LLNSRTRKNY</u></b>LAWYQ QKPGQSPKLLIY<b><u>WAST</u></b> RESGVPDRFTGSGSGT DFTLTISSVQAE DLAV YYC<b><u>KQSYNLYT</u></b>FGGG TKLEIK</p>
<p>301-03 Kappa 2 SEQ ID NO: 88, 89</p>	<p>ATGAAGTTGCTGTAGGCTGTTGGTGTGATGTTCTGGATTCCCT GCTTCCAGCAGTGTGTTTTGATGACCCAACTCCACTCTCCCTG CCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTCAGATCTAGT <b><u>CAGAGCATTGTACATAGTAATGGAACACCTAT</u></b>TTAGAATGGTAC CTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTAC<b><u>AAAGTT</u></b> <b><u>TCC</u></b>AACCGATTTCTGGGGTCCAGACAGGTTCACTGGCAGTGG TCAGGGACAGATTTCACTCAAGATCAGCAGAGTGGAGGCTGAG GATCTGGGAGTTTATTCTGCT<b><u>TTTCAAGTTTACATGTTCTCTCT</u></b> <b><u>ACG</u></b>TTCCGGTGTGGGACCAAGCTGGAGCTGAAA</p>	<p>MKLPVRLVLMFWIPA SSSDVLMQTPLSLPV SLGDQASISCRSS<b><u>QSI</u></b> <b><u>VHSGNTY</u></b>LEWYLQKP GQSPKLLIY<b><u>KVSNRFS</u></b> GVPDRFSGSGSDFT LKISRVEAEDLGVYFC <b><u>FQGSHPVLT</u></b>FGAGTKL ELK</p>
<p>301-17 IgG3 SEQ ID NO: 96, 97</p>	<p>ATGGGATGGAGCTGTATCATCCTCTTTTGGTAGCAACAGCTACA GGTGTCCACTCCAGGTCCTCACTGCAGCAGCTGGGGCTGAGCTT GTGAAGCCTGGGGCTTCAAGTAAATGCTGCAAGGCTTCT<b><u>TGGC</u></b> <b><u>TACAGCTTCCACAGCTACTGG</u></b>ATAAACTGGGTGAAGCAGAGGCCT GGACAAGGCCTTGAGTGGATTGGAGAT<b><u>GTTTCATCCTGGTAGAGGT</u></b> <b><u>GTTTCTACC</u></b>TACAATGCCAAGTTCAAGAGCAAGGCCACACTGACT CTAGACAGTCTCCAGCACAGCCTACATGCAGCTCAGCAGCCTG ACATCTGAGGACTCTGCGGTCTATTATTG<b><u>TCAAGATCCACGGT</u></b> <b><u>AATACCTACTGGTTCTTCGATGTC</u></b>TGGGGCGCAGGGACCACGGTC ACCGTCTCCTCAGCTACAACAACAGCCCATCT</p>	<p>MGWSCIILFLVATATG VHSQVQLQPPGAE LVK PGASVKMSCKAS<b><u>GYSF</u></b> <b><u>TSYWI</u></b>NWVKQRFPGQGL EWIGD<b><u>VHPRGRVST</u></b>YN AKFKSKATLTLDTSSS TAYMQLSSLTSEDSAV YYC<b><u>SRSHGNTYWF</u></b>FDV WGAGTFTVVSATTTA PS</p>
<p>301-17 Kappa</p>	<p>ATGAAGTTGCTGTAGGCTGTTGGTGTGATGTTCTGGATTCCCT GCTTCCAGCAGTGTGTTTTGATGACCCAACTCCACTCTCCCTG CCTGTCAGTCTTGGAGATCAAGCCTCCATCTCTTCAGATCTAGT <b><u>CAGAGCATTGTACATAGTAATGGAACACCTAT</u></b>TTAGAATGGTAC CTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAA<b><u>AGTT</u></b></p>	<p>MKLPVRLVLMFWIPA SSSDVLMQTPLSLPV SLGDQASISCRSS<b><u>QSI</u></b> <b><u>VHSGNTY</u></b>LEWYLQKP GQSPKLLIY<b><u>KVSNRFS</u></b></p>

SEQ ID NO: 98, 99	<b><u>TC</u>CAACCGATTTTCTGGGGTCCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGATTTACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGCT<b><u>TTTCAAGGTTACATGTTCCATTC</u></b><b><u>ACG</u></b>TTTCGGCTCGGGGACAAAGTTGGAAATAAAACGGGCTGATGCT</b>	GVPDRFSGSGSGTDFTLKISRVEAEDLGVYYCF <b><u>FGSHVPFT</u></b> FGSGTKLEIKRADA
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Table 4A

Chimeric/Humanized Antibody 301-11
VH0* SEQ ID NO: 13, 14
VH1 SEQ ID NO: 15, 16
VH2 SEQ ID NO: 17, 18
VH3 SEQ ID NO: 19, 20
VH4 SEQ ID NO: 21, 22
VH5 SEQ ID NO: 23, 24
VH6 SEQ ID NO: 25, 26
VK0* SEQ ID NO: 27, 28
VK1 SEQ ID NO: 29, 30
VK2 SEQ ID NO: 31, 32
VK3 SEQ ID NO: 33, 34
VK4 SEQ ID NO: 35, 36
VK5 SEQ ID NO: 37, 38
VK6 SEQ ID NO: 39, 40

Table 4B

Humanized Antibody	cDNA Sequence	Polypeptide Sequence
301-17 VH0* SEQ ID NO: 41, 42	CAGGTCCAACCTGCAGCAGCCTGGGGCTGAGCTTGTGAAGCCTGGG GCTTCAGTGAAGATGTCTGCAAGGCTTCT <b><u>GGCTACAGCTTCACC</u></b> <b><u>AGCTACTGG</u></b> AATAAAGTGGGTGAAGCAGAGGCTGGACAAGGCCTT GAGTGGATTGGAGAT <b><u>GTGCATCCTGGTAGAGGCGTGTCCACA</u></b> TAC AATGCTAAGTTCAAGAGCAAGGCCACACTGACTCTGGACACATCC TCCAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC TCTGCGGTCTATTACTGT <b><u>AGCAGATCCCATGGTAACACCTACTGG</u></b> <b><u>TTTTTTGACGTC</u></b> TGGGGCGCAGGCCACCACGGTCACCGTCTCCTCA	QVQLQQPGAELVKPGA SVKMSCKAS <b><u>GYSFTSY</u></b> <b><u>WINVWKQRPQGLEWI</u></b> GD <b><u>VHPGRGVST</u></b> YNAKF KSKATLTLDTSSTAY MQLSSLTSEDSAVYYC <b><u>SRSHGNTYWFFDV</u></b> WGA GTTVTVSS
VH1 SEQ ID NO: 43, 44	CAGGTCCAACCTGGTGCAGTCTGGGGCTGAGCTTAAGAAGCCTGGG GCTTCAGTGAAGATGTCTGCAAGGCTTCT <b><u>GGCTACAGCTTCACC</u></b> <b><u>AGCTACTGG</u></b> AATAAAGTGGGTGAAGCAGAGGCTGGACAAGGCCTT GAGTGGATTGGAGAT <b><u>GTGCATCCTGGTAGAGGCGTGTCCACA</u></b> TAC AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGCAGCTCAGCAGCCTGACATCTGAGGAC TCTGCGGTCTATTACTGT <b><u>AGCAGATCCCATGGTAACACCTACTGG</u></b> <b><u>TTTTTTGACGTC</u></b> TGGGGCCAAGGCCACCACGGTCACCGTCTCCTCA	QVQLVQSGAELKKPGA SVKMSCKAS <b><u>GYSFTSY</u></b> <b><u>WINVWKQRPQGLEWI</u></b> GD <b><u>VHPGRGVST</u></b> YNAKF KSRATLTLDTSISTAY MQLSSLTSEDSAVYYC <b><u>SRSHGNTYWFFDV</u></b> WGQ GTTVTVSS

<p>VH2 SEQ ID NO: 45, 46</p>	<p>CAGGTCCAAC TGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGATGTCTTCAAGGCTTCT<b>GGCTACAGCTTCACC</b> <b>AGCTACTGG</b>AATAAAGTGGGTGAAGCAGAGGCTTGGACAAGGCCTT GAGTGGATTGGAGAT<b>GTGCATCCTGGTAGAGCGGTGTCCACATAC</b> AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT<b>AGCAGATCCCATGGTAACACCTACTGG</b> <b>TTTTTTGACGTC</b>TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA</p>	<p>QVQLVQS GAEVKKPGA SVKMSCKAS<b>GYSFTSY</b> <b>WINWVKRQ</b>RPQGLEWI GD<b>VHPGRGVST</b>YNAKF KSRATLTLDTSTAY MELSSLRSEDTAVYYC <b>SRSHGNTYWFEDV</b>WGQ GTTVTVSS</p>
<p>VH3 SEQ ID NO: 47, 48</p>	<p>CAGGTCCAAC TGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCTTCAAGGCTTCT<b>GGCTACAGCTTCACC</b> <b>AGCTACTGG</b>AATAAAGTGGGTGCGACAGAGGCTTGGACAAGGCCTT GAGTGGATTGGAGAT<b>GTGCATCCTGGTAGAGCGGTGTCCACATAC</b> AATGCTAAGTTCAAGAGCAGAGCCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT<b>AGCAGATCCCATGGTAACACCTACTGG</b> <b>TTTTTTGACGTC</b>TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA</p>	<p>QVQLVQS GAEVKKPGA SVKVSCKAS<b>GYSFTSY</b> <b>WINWVRQ</b>RPQGLEWI GD<b>VHPGRGVST</b>YNAKF KSRATLTLDTSTAY MELSSLRSEDTAVYYC <b>SRSHGNTYWFEDV</b>WGQ GTTVTVSS</p>
<p>VH4 SEQ ID NO: 49, 50</p>	<p>CAGGTCCAAC TGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCTTCAAGGCTTCT<b>GGCTACAGCTTCACC</b> <b>AGCTACTGG</b>AATAAAGTGGGTGCGACAGAGGCTTGGACAAGGCCTT GAGTGGATTGGAGAT<b>GTGCATCCTGGTAGAGCGGTGTCCACATAC</b> AATGCTAAGTTCAAGAGCAGAGTCACACTGACTCTGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT<b>AGCAGATCCCATGGTAACACCTACTGG</b> <b>TTTTTTGACGTC</b>TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA</p>	<p>QVQLVQS GAEVKKPGA SVKVSCKAS<b>GYSFTSY</b> <b>WINWVRQ</b>RPQGLEWI GD<b>VHPGRGVST</b>YNAKF KSRVTLTLDTSTAY MELSSLRSEDTAVYYC <b>SRSHGNTYWFEDV</b>WGQ GTTVTVSS</p>
<p>VH5 SEQ ID NO: 51, 52</p>	<p>CAGGTCCAAC TGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCTTCAAGGCTTCT<b>GGCTACAGCTTCACC</b> <b>AGCTACTGG</b>AATAAAGTGGGTGCGACAGAGGCTTGGACAAGGCCTT GAGTGGATTGGAGAT<b>GTGCATCCTGGTAGAGCGGTGTCCACATAC</b> AATGCTAAGTTCAAGAGCAGAGTCACACTGACTAGGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT<b>AGCAGATCCCATGGTAACACCTACTGG</b> <b>TTTTTTGACGTC</b>TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA</p>	<p>QVQLVQS GAEVKKPGA SVKVSCKAS<b>GYSFTSY</b> <b>WINWVRQ</b>RPQGLEWM GD<b>VHPGRGVST</b>YNAKF KSRVTLTRDTSISTAY MELSSLRSEDTAVYYC <b>SRSHGNTYWFEDV</b>WGQ GTTVTVSS</p>
<p>VH6 SEQ ID NO: 53, 54</p>	<p>CAGGTCCAAC TGGTGCAGTCTGGGGCTGAGGTGAAGAAGCCTGGG GCTTCAGTGAAGGTGTCTTCAAGGCTTCT<b>GGCTACAGCTTCACC</b> <b>AGCTACTGG</b>AATAAAGTGGGTGCGACAGAGGCTTGGACAAGGCCTT GAGTGGATTGGAGAT<b>GTGCATCCTGGTAGAGCGGTGTCCACATAC</b> AATGCTAAGTTCCAGGGCAGAGTCACAATGACTAGGGACACATCC ATAAGCACAGCCTACATGGAGCTCAGCAGCCTGAGATCTGAGGAC ACGGCGGTCTATTACTGT<b>AGCAGATCCCATGGTAACACCTACTGG</b> <b>TTTTTTGACGTC</b>TGGGGCCAAGGCACCACGGTCACCGTCTCCTCA</p>	<p>QVQLVQS GAEVKKPGA SVKVSCKAS<b>GYSFTSY</b> <b>WINWVRQ</b>RPQGLEWM GD<b>VHPGRGVST</b>YNAKF QGRVMTTRDTSISTAY MELSSLRSEDTAVYYC <b>SRSHGNTYWFEDV</b>WGQ GTTVTVSS</p>
<p>VK0* SEQ ID NO: 55, 56</p>	<p>GATGTTTTGATGACCCAATCTCCACTCTCCCTGCCTGTCAGTCTT GGAGATCAAGCCTCCATCTCTTGCAGATCTAGT<b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b>TTAGAATGGTACCTGCAGAAACCA GGCCAGTCTCCAAGCTCCTGATCTAC<b>AAAGTTTCCA</b>AACCGATTT TCTGGGGTCCCAGACAGGTTTCAAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATCTGGGAGTT TATTACTGC<b>TTTCAAGGTTACATGTTCTTCACT</b>TTTTGGCAGC GGGACCAAGCTGGAGATCAAA</p>	<p>DVLMTQT PLSLPVSLG DQASISCRSS<b>QSIVHS</b> <b>NGNTY</b>LEWYLQKPGQS PKLLIY<b>KVS</b>NRFSGVP DRFSGSGSDFTLKI SRVEAEDLVYYC<b>FOG</b> <b>SHVPFT</b>FGSGTKLEIK</p>
<p>VK1 SEQ ID NO: 57, 58</p>	<p>GATGTTTTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCTCCATCTCTTGCAGATCTAGT<b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b>TTAGAATGGTTTCAGCAGAAACCA GGCCAGTCTCCAAGGCGCCTGATCTAC<b>AAAGTTTCCA</b>AACCGATTT TCTGGGGTCCCAGACAGGTTTCAAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC<b>TTTCAAGGTTACATGTTCTTCACT</b>TTTTGGCCAA GGGACCAAGCTGGAGATCAAA</p>	<p>DVLMTQS PLSLPVTLG QPASISCRSS<b>QSIVHS</b> <b>NGNTY</b>LEWFQKPGQS PRRLIY<b>KVS</b>NRFSGVP DRFSGSGSDFTLKI SRVEAEDLVYYC<b>FOG</b> <b>SHVPFT</b>FGQGTKLEIK</p>
<p>VK2 SEQ ID NO:</p>	<p>GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCTT GGACAGCCGGCTCCATCTCTTGCAGATCTAGT<b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b>TTAGAATGGTTTCAGCAGAAACCA GGCCAGTCTCCAAGGCGCCTGATCTAC<b>AAAGTTTCCA</b>AACCGATTT</p>	<p>DVVMTQS PLSLPVTLG QPASISCRSS<b>QSIVHS</b> <b>NGNTY</b>LEWFQKPGQS PRRLIY<b>KVS</b>NRFSGVP</p>

59, 60	TCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC <b>TTCAAGGTTACATGTTCCCTTTCACT</b> TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DRFSGSGSGTDFTLKI SRVEAEDVGVYYC <b>FOG</b> <b>SHVPFT</b> FGQGTKLEIK
VK3 SEQ ID NO: 61, 62	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT <b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b> TTAGAATGGTTTCAGCAGAGGCCA GGCCAGTCTCCAAGGCGCCTGATCTAC <b>AAAGTTTCCA</b> AACCGATTT TCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC <b>TTCAAGGTTACATGTTCCCTTTCACT</b> TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQS PLSL PVTLG QPASISCRSS <b>QSIVHS</b> <b>NGNTY</b> LEWFQORPGQS PRLLIY <b>KVSNR</b> FSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC <b>FOG</b> <b>SHVPFT</b> FGQGTKLEIK
VK4 SEQ ID NO: 63, 64	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT <b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b> TTAGAATGGTACCTGCAGAGGCCA GGCCAGTCTCCAAGGCTGCTGATCTAC <b>AAAGTTTCCA</b> AACCGATTT TCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC <b>TTCAAGGTTACATGTTCCCTTTCACT</b> TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQS PLSL PVTLG QPASISCRSS <b>QSIVHS</b> <b>NGNTY</b> LEWYLQORPGQS PKLLIY <b>KVSNR</b> FSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC <b>FOG</b> <b>SHVPFT</b> FGQGTKLEIK
VK5 SEQ ID NO: 65, 66	GATGTTCTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT <b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b> TTAGAATGGTACCAGCAGAGGCCA GGCCAGTCTCCAAGGCTGCTGATCTAC <b>AAAGTTTCCA</b> AACCGATTT TCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC <b>TTCAAGGTTACATGTTCCCTTTCACT</b> TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVLMTQS PLSL PVTLG QPASISCRSS <b>QSIVHS</b> <b>NGNTY</b> LEWYQORPGQS PRLLIY <b>KVSNR</b> FSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC <b>FOG</b> <b>SHVPFT</b> FGQGTKLEIK
VK6 SEQ ID NO: 67, 68	GATGTTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACCCCTT GGACAGCCGGCCTCCATCTCTTGCAGATCTAGT <b>CAGAGCATTGTA</b> <b>CATAGTAATGGAAACACCTAT</b> TTAGAATGGTACCAGCAGAGGCCA GGCCAGTCTCCAAGGCTGCTGATCTAC <b>AAAGTTTCCA</b> AACCGATTT TCTGGGGTCCAGACAGGTTTCAGTGGCAGTGGATCAGGGACAGAT TTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGTTGGAGTT TATTACTGC <b>TTCAAGGTTACATGTTCCCTTTCACT</b> TTTGGCCAA GGGACCAAGCTGGAGATCAAA	DVVMTQS PLSL PVTLG QPASISCRSS <b>QSIVHS</b> <b>NGNTY</b> LEWYQORPGQS PRLLIY <b>KVSNR</b> FSGVP DRFSGSGSGTDFTLKI SRVEAEDVGVYYC <b>FOG</b> <b>SHVPFT</b> FGQGTKLEIK

- 5 \*VH0 and VK0 denotes chimeric antibodies comprised of the human constant domain and mouse variable domain sequences

Table 5 Humanized antibody IgG4 sequence

Constant regions	cDNA Sequence	Polypeptide sequence
IgG4 heavy chain SEQ ID NO: 69, 70	GCTTCCACCAAGGGCCCATCCGTCTTCCCCCTGGCGCCCTGCTCC AGGAGCACCTCCGAGAGCACAGCCGCCCTGGCTGCCTGGTCAAG GACTACTTCCCCGAACCGGTGACGGTGTCTGGAACTCAGGCGCC CTGACCAGCGCGTGCACACCTTCCCGGCTGTCTACAGTCTCA GGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCTCCAGCAGC TTGGGCACGAAGACCTACACCTGCAATGTAGATCACAAGCCAGC AACACCAAGGTGGACAAGAGAGTTGAGTCCAATATGGTCCCCCA TGCCACCATGCCAGCACCTGAGTTCTGGGGGACCATCAGTC TTCTGTTCCCCCAAAACCAAGGACACTCTCATGATCTCCCGG ACCCCTGAGGTCACGTGCGTGGTGGTGGACGTGAGCCAGGAAGAC CCCGAGGTCCAGTTCAACTGGTACGTGGATGGCTGGAGGTGCAT AATGCCAAGACAAAGCCGCGGGAGGAGCAGTTCAACAGCACGTAC CGTGTGGTCAGCGTCTCACCGTCTGCACCAGGACTGGCTGAAC GGCAAGGAGTACAAGTGCAAGGTCCTCAACAAAGGCCTCCCGTCC TCCATCGAGAAAACATCTCCAAGGCCAAAGGGCAGCCCCGAGAG	ASTKGPSVFPLAPCSR STSESTAALGCLVKDY FPEPVTVSWNSGALTS GVHTFPAVLQSSGLYS LSSVTVPSSSLGKT YTCNVDPKPSNTKVDK RVESKYGPPCPPECPAP EFLGGPSVFLFPPKPK DTLMISRTPEVTCVVV DVSQEDPEVQFNWYVD GVEVHNAKTKPREEQF NSTYRVVSVLTVLHQD WLNKEYKCKVSNKGL PSSIEKTIKAKGQPR EPQVYITLPPSQEEMTK

	CCACAGGTGTACACCCTGCCCCATCCCAGGAGGAGATGACCAAG AACCAGGTGAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGC GACATGCCCGTGGAGTGGGAGAGCAATGGGCAGCCGGAGAACAAC TACAAGACCACGCCCTCCCGTGGACTCCGACGGCTCCTTCTTC CTCTACAGCAGGCTAACCGTGGACAAGAGCAGGTGGCAGGAGGGG AATGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACAACCAC TACACACAGAAGAGCCTCTCCCTGTCTCTGGGTAATGA	NQVSLTCLVKGFYPSD IAVEWESNGQPENNYK TTPFVLDSDGSFFLYS RLTVDKSRWQEGNVFS CSVMHEALHNHYTQKS LSLSLGK
<b>Kappa</b> SEQ ID NO: 71, 72	CGAACTGTGGCTGCACCATCTGTCTTCATCTTCCGCCATCTGAT GAGCAGTTGAAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAAT AACTTCTATCCAGAGAGGCCAAAGTACAGTGAAGGTGGATAAC GCCCTCCAATCGGGTAACTCCAGGAGAGTGTACAGAGCAGGAC AGCAAGGACAGCACCTACAGCCTCAGCAGCACCTGACGCTGAGC AAAGCAGACTACGAGAAACACAAAGTCTACGCCTGCGAAGTCACC CATCAGGGCTGAGCTCGCCCGTCAAAAGAGCTTCAACAGGGGA GAGTGTAG	RTVAAPSVFIFPPSDE QLKSGTASVVCLLNMF YPREAKVQWKVDNALQ SGNSQESVTEQDSKDS TYSLSSTLTLSKADYE KHKVYACEVTHQGLSS PVTKS FNRGEC

5

**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-amyloid $\beta$  6E10, all purchased from Biologend, and purified antibodies 301-11 and 301-17. All antibodies were used at 1  $\mu$ g/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 (70,000xg for 90 min). Supernatants were collected, aliquoted and stored at -80°C.

5 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.

10 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. 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**

20 [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.

25 [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.

30

**Example 3**

**Binding to A-beta synthetic oligomers.**

5 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).

[00282] 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**

15 Immunohistochemistry on Formalin Fixed Tissues

[00283] 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 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.

[00284] 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**

35 **Recombinant IgG1 and IgG2a antibodies**

5 [00285] 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.

[00286]

**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 GCCTCCGTGAAGATGTCTTGCAAGGCCCTCCGGCTACTCCTTCACC AGCTACTGGATCAACTGGGTGAAGCAGAGGCCGGACAGGGCCTG GAGTGGATTGGAGACGTGCACCCTGGCCGGGAGTGTCCACCTAC AACGCCAAGTTC AAGTCCAAGGCCACCTGACCCTGGACACCTCC AGCTCCACCGCTACATGCAGCTGTCTCCCTGACCCTCCGAGGAC TCCGCCGTGTACTACTGCAGCAGGTCCCACGGCAACACCTACTGG TTTTTCGACGTGTGGGGCGCCGAACCACAGTGACCGTGTCTCTCC GCCAAAACGACACCCCATCTGTCTATCCACTGGCCCTGGATCT GCTGCCAAACTAACTCCATGGTACCCTGGGATGCCTGGTCAAG GGCTATTTCCCTGAGCCAGTGACAGTGACCTGGAACCTCTGGATCC CTGTCCAGCGGTGTGCACACCTTCCCAGCTGTCTGGAGTCTGAC CTCTACACTCTGAGCAGCTCAGTGACTGTCCCTCCAGCCCTCGG CCCAGCGAGACCGTCACCTGCAACGTTGCCACCCGGCCAGCAGC ACCAAGGTGGACAAGAAAATTGTGCCAGGGATTGTGGTTGTAAG CCTTGCATATGTACAGTCCAGAAGTATCATCTGTCTTCATCTTC CCCCCAAAGCCCAAGGATGTGCTCACCATTACTCTGACTCCTAAG GTCACGTGTGTGTGGTAGACATCAGCAAGGATGATCCCAGGGT CAGTCCAGCTGGTTTGTAGATGATGTGGAGGTGCACACAGCTCAG ACGCAACCCCGGAGGAGCAGTTCAACAGCACTTTCCGCTCAGTC AGTGAACCTCCCATCATGCACCAGGACTGGCTCAATGGCAAGGAG TTCAAATGCAGGGTCAACAGTGCAGCTTTCCCTGCCCCATCGAG AAAACCATCTCCAAAACCAAGGCAGACC GAAGGCTCCACAGGTG TACACCATCCACCTCCCAAGGAGCAGATGGCCAAGGATAAAGTC AGTCTGACCTGCATGATAACAGACTTCTTCCCTGAAGACATTACT GTGGAGTGGCAGTGGAAATGGGCAGCCAGCGGAGAACTACAAGAAC ACTCAGCCCATCATGAACACGAATGGCTCTTACTTTCGTCTACAGC AAGTCAAATGTGCAGAAGAGCAACTGGGAGGCAGGAAATACTTTTC ACCTGCTCTGTGTTACATGAGGGCTGCACAACCACCACTACTGAG AAGAGCCTCTCCACTCTCCTGGTAAATGATGA	QVQLQQPGAELVKPGA SVKMSCKASGYSFTSY WINWVKQRPQGLEWI GDVHPGRGVSTYNAKF KSKATLTLDTSSSTAY MQLSSLTSEDSAVYYC SRSHGNTYWFVDVWGA GTTVTVSSAKTTPPSV YPLAPGSAQTNSMVT LGCLVKGYFPEPVTVT WNSGSLSSGVHTFPAV LES DLYTLSSSVTVPS SPRPSETVTCNVAHPA SSTKVDKKIVPRDCGC KPCICTVPEVSVVFI PPKPKDVLITLTPKV TCVVVDISKDDPEVQF SFWVDDVEVHTAQTQP REEQFNSTFRSVSELP IMHQDWLNGKEFKCRV NSAAFPAPIEKTISK KGRPKAPQVYTIPTPK EQMAKDKVSLTCMITD FFPEDITVEWQWNGQP AENYKNTQPIMNTNGS YFVYSKLNQKSNWEA GNTFTCSVLHEGLHNH HTEKSLSHSPGK
301-17 IgG2a SEQ ID NO: 92, 93	CAGGTGCAGCTGCAGCAGCCTGGCGCTGAGCTGGTGAAGCCTGGA GCCTCCGTGAAGATGTCTTGCAAGGCCCTCCGGCTACTCCTTCACC AGCTACTGGATCAACTGGGTGAAGCAGAGGCCGGACAGGGCCTG GAGTGGATTGGAGACGTGCACCCTGGCCGGGAGTGTCCACCTAC AACGCCAAGTTC AAGTCCAAGGCCACCTGACCCTGGACACCTCC AGCTCCACCGCTACATGCAGCTGTCTCCCTGACCCTCCGAGGAC TCCGCCGTGTACTACTGCAGCAGGTCCCACGGCAACACCTACTGG TTTTTCGACGTGTGGGGCGCCGAACCACAGTGACCGTGTCTCTCC GCCAAAACAACAGCCCATCGGTCTATCCACTGGCCCTGTGTGT GGAGATAACAACAGCTCCTCGGTGACTCTAGGATGCCTGGTCAAG GGTTATTTCCCTGAGCCAGTGACCTTGACCTGGAACCTCTGGATCC CTGTCCAGTGGTGTGCACACCTTCCCAGCTGTCTGCAGTCTGAC CTCTACACCTCAGCAGCTCAGTGACTGTAACCTCGAGCACCTGG CCCAGCCAGTCCATCACCTGCAATGTGGCCACCCGGCAAGCAGC ACCAAGGTGGACAAGAAAATTGAGCCAGAGGGCCACCAATCAAG CCCTGCTCCATGCAAAATGCCAGCACCTAACCTCTTGGGTGGA CCATCCGTCTCATCTTCCCTCCAAAGATCAAGGATGTAATCATG ATCTCCCTGAGCCCATAGTCACATGTGTGGTGGTGGATGTGAGC	QVQLQQPGAELVKPGA SVKMSCKASGYSFTSY WINWVKQRPQGLEWI GDVHPGRGVSTYNAKF KSKATLTLDTSSSTAY MQLSSLTSEDSAVYYC SRSHGNTYWFVDVWGA GTTVTVSSAKTTPPSV YPLAPVCGDITGSSVT LGCLVKGYFPEPVTLT WNSGSLSSGVHTFPAV LQSDLYTLSSSVTVTS STWPSQSTCNVAHPA SSTKVDKKIEPRGPTI KPCPPCKPAPNLLGG PSVFIPTPKIKDVLMI SLSPIVTCVVVDVSED DPDVQISWVNNVEVH

	GAGGATGACCCAGATGTCCAGATCAGCTGGTTTGTGAACAACGTG GAAGTACACACAGCTCAGACACAAACCCATAGAGAGGATTACAAC AGTACTCTCCGGGTGGTCAGTGCCCTCCCCATCCAGCACCAGGAC TGGATGAGTGGCAAGGAGTTCAAATGCAAGGTCAACAACAAGAC CTCCCAGCGCCCATCGAGAGAACCATCTCAAACCCAAAGGGTCA GTAAGAGCTCCACAGGTATATGTCTTGCCCTCCACCAGAAGAAGAG ATGACTAAGAAACAGGTCACCTCTGACCTGCATGGTCACAGACTTC ATGCCTGAAGACATTTACGTGGAGTGGACCAACAACGGGAAAACA GAGCTAAACTACAAGAACACTGAACCAGTCTGGACTCTGATGGT TCTTACTTTCATGTACAGCAAGCTGAGAGTGGAAAAGAAGAACTGG GTGGAAGAATAAGTACTCTCTGTTTCAGTGGTCCACGAGGGTCTG CACAATCACCACACGACTAAGAGCTTCTCCCGGACTCCGGGTAAA TGATGA	TAQTQTHREDYNSTLR VVSALPIQHQDWMSGK EFKCKVNNKDLPAPIE RTISKPKGSVRAPQVY VLPPPEEEMTKKQVTL TCMVTDMPEDIYVEW TNNGKTELNKNTPEV LSDGSYFMYSKLRVE KKNWVERNSYSCSVVH EGLHNHHTTKSFSRTP GK
301-17 Kappa SEQ ID NO: 94, 95	GATGTGCTGATGACCCAGACCCCTCTGTCCCTGCCTGTGTCCCTG GGCGATCAGGCCAGCATCTCCTGCAGGTCTCCAGTCCATCGTG CACTCCAACGGCAACACCTACCTGGAGTGGTACCTGCAGAAGCCC GGCCAGTCCCCAAGCTGCTGATCTACAAGGTGTCCAACCGGTTT TCCGGCGTGCCGATAGGTTCTCCGGATCCGGCTCCGGCACCGAC TTTACCCTGAAGATCTCCAGGGTGGAGGCCGAGGACCTGGGCGTG TACTACTGCTTTCAGGGCTCCCACGTGCCCTTCACCTTCGGCTCC GGCACCAGCTGGAGATCAAGCGGGCTGATGCTGCACCAACTGTA TCCATCTTCCCACCATCCAGTGAGCAGTTAACATCTGGAGGTGCC TCAGTCGTGTCTTCTTGAACAACCTTCTACCCCAAAGACATCAAT GTCAAGTGGAAAGATTGATGGCAGTGAACGACAAAATGGCGTCTTG AACAGTTGGACTGATCAGGACAGCAAAGACAGCACCTACAGCATG AGCAGCACCTCACGTTGACCAAGGACGAGTATGAACGACATAAC AGCTATACCTGTGAGGCCACTACAAGACATCAACTTCACCCATT GTCAAGAGCTTCAACAGGAATGAGTGTGATGA	DVLMTQTPLSLPVSIG DQASISCRSSQSIVHS NGNTYLEWYLQKPGQS PKLLIYKVSNRFSGVP DRFSGSGSDFTLKI SRVEAEDLGYYCFQG SHVPFTFGSGTKLEIK RADAAPTVSIFPPSSE QLTSGGASVVCFLNPF YPKDINVWKIDGSER QNGVLSWTDQDSKDS TYSMSSTLTLTKDEYE RHNSYTCEATHKTS PIVKSFNHNEC

5

**[00287]** The recombinant 301-17 IgG1 and IgG2a antibodies were tested and compared to the parent hybridoma-purified IgG3 antibody for binding characteristics as described below.

**[00288]** 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:**

**[00289]** Approximate kinetic values were:

Hybridoma: KD = 26.9 nM

15 IgG2a-301-17 antibody: KD = 16.2 - 19.5 nM No binding was detected with control mouse IgG.

**[00290]** 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, Biologend) and rabbit anti-Abeta antibodies (D54D2, Cell Signaling; ab201060, (abcam; NBP1-78007, Novus).

20

5 [00291] *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  $\mu$ M. 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.

10 [00292] *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: 12 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-A $\beta$ 6E10 antibody (Biolegend)

## 15 Example 6

### Inhibition of Oligomer Propagation

[00293] The biological functionality of antibodies was tested in vitro by examining their effects on Amyloid Beta (A $\beta$ ) aggregation using the Thioflavin T (ThT) binding assay. A $\beta$  aggregation is induced by and propagated through nuclei of preformed small A $\beta$  oligomers, and the complete process from monomeric A $\beta$  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, A $\beta$  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 A $\beta$ 1-42 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.

[00294] A $\beta$ 42 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 A $\beta$  molecular species are at equilibrium and during which there is no increase in fluorescence. Co-incubation of A $\beta$ 42 with an irrelevant mouse antibody did not have any significant effect on the aggregation process. In contrast, co-incubation of A $\beta$ 42 with the test antibodies completely inhibited all phases of the aggregation process. Results obtained with antibody 301-11 are shown in FIG. 1.

[00295] Near identical results were obtained with 301-17 as well as 301-3.

40 [00296] As the ThT aggregation assay mimics the in vivo biophysical / biochemical stages of A $\beta$  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.

5 **Example 7****Toxicity inhibition assay**

[00297] The inhibition of toxicity of A-beta42 oligomers by antibodies can be tested in a rat primary cortical neuron assay.

[00298] 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.

[00299] An exemplary set up is shown in Table 9.

[00300] 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**

A $\beta$ O / MAB molar ratio	A $\beta$ O ( $\mu$ L)	A $\beta$ O ( $\mu$ M)	AB ( $\mu$ M)	AB ( $\mu$ L)	Medium ( $\mu$ L)	Final volume ( $\mu$ L)
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

A $\beta$ O working solution: 2,2 mg/mL - 500 $\mu$ M
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CTRL vehicle:	1,68 $\mu$ L of oligomer buffer + 127,3 $\mu$ L PBS + 711 $\mu$ L culture medium
CTRL A $\beta$ O:	1,68 $\mu$ L of A $\beta$ O + 127,3 $\mu$ L PBS + 711 $\mu$ L culture medium
CTRL HNG:	1,68 $\mu$ L of A $\beta$ O + 8,4 $\mu$ L HNG (100 nM final) + 127,3 $\mu$ L PBS + 702,6 $\mu$ L culture medium

20

[00301] 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

25 **Example 8****In vivo toxicity inhibition assay**

[00302] The inhibition of toxicity of A-beta42 oligomers by the antibodies can be tested in vivo in mouse behavioral assays.

**Novel Object Recognition (NOR)**

5 **[00303]** 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 entorhinal 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:

15 **[00304]** 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 A $\beta$  oligomerization) or A $\beta$ O (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.

20 **[00305]** 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 A $\beta$ O in the absence or presence of antibody in a total volume of 5  $\mu$ L. The experimental groups were defined as follow:

- GROUP A (vehicle CTRL): ICV injection of vehicle (n = 12)
- GROUP B (A $\beta$ O CTRL): ICV injection of A $\beta$ O (n = 12)
- 25 • GROUP C (Antibody CTRL): ICV injection of A $\beta$ O + antibody (n = 12)
- GROUP D (Treatment): ICV injection of A $\beta$ O + antibody (n = 12)

30 **[00306]** Before ICV injection, 4  $\mu$ L of antibody 1 (*i.e.* 112 pmoles) were incubated for 30 minutes at room temperature with 1  $\mu$ L vehicle (*i.e.* buffer for A $\beta$  oligomerization) or 1  $\mu$ L A $\beta$ O (50 pmoles) corresponding to an antibody/A $\beta$ O molar ratio of 2.24.

**[00307]** At day 0, mice received a single 5  $\mu$ L ICV injection of vehicle or A $\beta$ O in the presence of vehicle or antibody.

35 **[00308]** 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

40

5 object) / total exploration time. If the total exploration time is  $\leq 5$  s, animals are excluded from the calculation of the discrimination index and statistical analysis.

[00309] Mice from the vehicle control group (Group A) exhibited normal behavior with a mean discrimination index of  $0.443 \pm 0.053$ . These results are in agreement with previous observations of similar control groups at SynAging. As expected, a single ICV injection of A $\beta$ O (Group B) resulted in a  
10 significant impairment ( $p < 0.0001$ ) of the cognitive performance when compared to vehicle control mice; with a mean discrimination index of  $-0.062 \pm 0.048$ . A $\beta$ O-injected mice were not able to discriminate between novel and familiar objects.

[00310] Mice dosed with antibody in the presence of vehicle (Group C) were found to exhibit normal cognitive performances with a mean discrimination index of  $0.439 \pm 0.049$ . These mice were  
15 not significantly different from vehicle control mice ( $p = 0.9163$ ) and significantly different from A $\beta$ O injected mice ( $p < 0.0001$ ).

[00311] When co-injected with A $\beta$ O, the antibody fully prevented A $\beta$ O-induced cognitive deficits in the NOR test. Indeed, mice from Group D exhibited a mean discrimination index of  $0.481 \pm 0.055$ , not different from control mice ( $p = 0.6126$ ) but different from A $\beta$ O-injected mice ( $p = 0.0002$ ). Taken  
20 together, the data suggest that antibody 301-17 offered protection against A $\beta$ O-induced cognitive deficits.

#### Synaptic Markers

[00312] 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.  
25 Hippocampi are collected, snap frozen and stored at  $-80^{\circ}\text{C}$  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  
30 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.

[00313] Brains are collected from mice that underwent the behavioral testing.

[00314] The hippocampus (relevant structure for memory formation) is dissected and  
35 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  $37^{\circ}\text{C}$ . and the supernatants are recovered after centrifuging.

[00315] 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).

40 [00316] 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.

5 **Example 9****In vivo propagation inhibition assay**

[00317] 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.

15 **Table 10 A-beta Sequences and compounds**

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

**Table 11**

20 Human A-beta 1-42

DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA (SEQ ID NO: 73)

**Example 12**

[00318] **Recombinant antibodies (HHQK)** Recombinant IgG1 and IgG2a 301-17 constructs were made by grafting the variable region of hybridoma-derived 301-17 onto a murine IgG1 or IgG2a backbone (WuXi, Biologics).

[00319] The 301-17 IgG1 and IgG2a antibodies were tested and compared to the parent hybridoma-purified IgG3 antibody for binding characteristics as described below.

[00320] 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:**

[00321] 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.

Recombinant 301-17 IgG1 had a similar KD to the IgG2a recombinant.

[00322] 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

5 to bind cyclic peptide (SEQ ID NO: 2) conjugated to BSA with an approximate KD of 17 pM (average of 3 tests). No or negligible binding was detected for other commercial A-beta antibodies tested (pan-Abeta 6E10, Biolegend) and rabbit anti-A-beta antibodies (D54D2, Cell Signaling; ab201060, (abcam; NBP1-78007, Novus).

[00323] 301-17 IgG1 MAAS-2 binding to AbO: Recombinant 301-17 IgG1 and hybridoma-  
10 purified 301-17 IgG3 were immobilized on MAAS-2 sensor chips and tested for binding to AbO (SynAging) at 1  $\mu$ M. 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.

[00324] 301-17 IgG1 MAAS-2 binding to cyclic peptide epitope: Recombinant 301-17 IgG1 was  
15 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)

## 20 Example 13

Humanized antibody binding in soluble brain extracts

### Methods

[00325] Pool of soluble brain extracts injected at 0.5ml/min through Superdex 75(10/300) HPLC  
column for 50 minutes and 0.25ml fractions collected:

25 [00326] Soluble human AD brain extracts were separated into LMW (<70kDa) and high  
molecular (HMW; >70kDa) fractions by size-exclusion chromatography (SEC). Antibody binding to SEC  
fractions was assessed by surface plasmon resonance (SPR).

[00327] Specifically, pooled fractions were concentrated and total protein concentration  
30 determined by BCA assay. Pooled fractions were diluted to 100ug/ml and injected over immobilized  
antibodies on sensor chip of MASS2.

### Results

[00328] SEC fractionation of soluble human AD brain extracts gave rise to a reproducible  
pattern with LMW peaks consistent with the presence of reportedly toxic dimers, tetramers and  
dodecamers. Humanized antibody of 301-17 (VH2 Vk5) (SEQ ID NO: 45, 46 and SEQ ID NO: 65, 66)  
35 was compared to the mouse monoclonal version (301-17; Table 3) and other A-beta antibodies.

[00329] As shown in FIG. 2, mouse monoclonal 301-17 and aducanumab show equivalent  
binding to the toxic oligomer-enriched LMW fraction of soluble human AD brain extract.

[00330] SPR analysis showed preferential binding of humanized 301-17 to the toxic oligomer-  
enriched LMW fraction compared to aducanumab and bapineuzumab. The binding response of  
40 humanized 301-17 for the LMW fraction was ~1.5-2 fold greater than that obtained with aducanumab.

5

**Example 14**

**[00331] Methods:** Frozen human AD brain sections were stained with humanized 301-17 and other Abeta-directed antibodies to evaluate the degree of binding to parenchymal Abeta plaque and vascular A beta deposits.

10 **[00332] Results:** Clear binding of parenchymal and vascular Abeta in AD brain was observed with aducanumab and bapineuzumab, consistent with the clinical occurrence of ARIA associated with these antibodies. By comparison, no immunoreactivity of Abeta deposits was observed with humanized 301-17 (VH2Vk5).

15 **[00333] Conclusions:** Results obtained with AD patient material (Ex 13 and 14) suggest that humanized 301-17 may achieve greater therapeutic potency compared to other A $\beta$ -directed antibodies due to: 1) Selective targeting of soluble toxic LMW oligomers, and 2) Reduced risk of ARIA allowing for safe administration of higher doses of antibody. This data is shown in Fig. 3

**Example 15**

20 A $\beta$  monomers and oligomers

**[00334]** Recombinant A $\beta$ 42 peptide (California Peptide, Salt Lake City UT, USA) was 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 100 $\mu$ M in distilled water (dH<sub>2</sub>O) and used immediately. Oligomers were prepared by  
25 diluting the 5mM DMSO peptide solution in phenol red-free F12 medium (Life Technologies Inc., Burlington ON, Canada) to a final concentration of 100 $\mu$ M and incubated for 24 hours at 4°C followed by immediate use or storage at -80C.

Brain extract

30 **[00335]** Brain tissues from 11 different human AD patients were obtained from the University of Maryland Brain and Tissue Bank and from Dr. Jiri Safar at Case Western Reserve University (Cleveland, Ohio, USA). The clinical diagnosis of AD was based on NINCDS-ADRDA criteria. Samples from frontal cortex were weighed and subsequently submersed in a volume of fresh, ice cold TBS buffer 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  
35 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 for 90 min. Supernatants (soluble extracts) were collected, aliquoted and stored at -80°C. The protein concentration was determined using a bicinchoninic acid (BCA) protein assay. Pools of brain extracts from 3-8 patients were used in each analysis.

40

## 5 Size exclusion chromatography

[00336] Pooled soluble brain extracts were injected at 0.5 ml/min through a Superdex 75 (10/300) HPLC column for 50 minutes and 0.25ml fractions were collected. Molecular weight (MW) markers were run separately. Protein peaks were monitored by absorbance at O.D. 280 nm. Fractions corresponding to a MW of ~8kDa to ~70kDa were pooled into a low molecular weight (LMW) fraction.

10 A $\beta$  monomers (MW ~4.5kDa) were excluded from the LMW fraction. Fractions corresponding to a MW of >70kDa to ~700kDa were pooled into a high molecular weight (HMW) fraction. The LMW and HMW fractions were concentrated and total protein concentration was determined in a BCA assay. The fractions were then diluted in PBS-EP, BSA (2mg/ml) buffer to 100  $\mu$ g /ml for surface plasmon resonance (SPR) analysis.

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### Measurement of total A $\beta$ and aggregated A $\beta$ in brain extract

[00337] The amount of aggregated A $\beta$  and total A $\beta$  (monomers and aggregates) in the LMW and HMW fractions of pooled human AD soluble brain extract (pool from 3 brains) were measured at QPS (Grambach, Austria). Total amounts of A $\beta$ 38, 40 and 42 were determined in a commercial immunosorbent assay (MSD, Rockville MD, USA) using peptide standards. Aggregated A $\beta$  levels were measured using the Amorfix Aggregated A $\beta$  Assay (A4). Briefly, aggregated A $\beta$  was separated from monomeric A $\beta$  via the matrix of the Amorfix Disaggregation Plate. While oligomers attach to the matrix, monomers are found in the flow through. After two washing steps, the attached A $\beta$  aggregates are monomerized and eluted with HFIP. Monomerized A $\beta$  in the HFIP eluate was dried under a fume hood until complete evaporation of HFIP and resuspended in assay buffer. Measurement of resolved monomerized A $\beta$  aggregates was carried out in the MSD assay for A $\beta$ 38, 40 and 42. The total protein concentration in the LMW and HMW brain fractions was measured by BCA and the results are expressed as pg A $\beta$  species per mg total protein.

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### Surface plasmon resonance analysis

30 [00338] Surface plasmon resonance measurements were performed using a Molecular Affinity Screening System (Sierra Sensors GmbH, Hamburg, Germany). Purified antibodies were immobilized on sensorchips. Preparations of A $\beta$  peptide monomers, synthetic A $\beta$  oligomers or pooled soluble human AD brain extracts (100  $\mu$ g/ml) were injected over the surfaces followed by a dissociation phase. Sensorgrams were double-reference subtracted. The SPR results shown were replicated in 2-8 independent studies.

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### Immunohistochemistry

[00339] Fresh frozen AD brain sections were exposed to antigen retrieval citrate buffer (Target Retrieval Solution, Dako, Santa Clara CA, USA) for 20 min and incubated in a humidified chamber with serum-free protein blocking reagent (Dako) for 1h to block non-specific staining. The sections were incubated overnight at 4°C with primary antibodies ( $\mu$ 301-17, hu 301-17, aducanumab,

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5 bapineuzumab, isotype controls) at 1 µg/ml and washed 3 times for 5 min in Tris-buffered saline containing 0.1% TritonX-100 (TBS-T) buffer. Secondary HRP-conjugated rabbit anti-human IgG (0.4 µg/ml; Abcam, San Francisco CA, USA) or sheep anti-mouse IgG (1:1000 dilution; GE Healthcare, Chicago IL, USA) antibodies were added to the sections and incubated for 1 hour, followed by 3 washes in TBS-T buffer. Secondary antibody was also added to sections that were not exposed to primary antibody as a negative control. The HRP enzyme substrate, biaminobezidine (DAB) chromogen reagent (Vector Laboratories, Burlingame CA, USA), was then added to the sections followed by rinsing with distilled water. The sections were counterstained with haematoxylin QS (Vector Laboratories, Burlingame CA, USA). The slides were examined under a light microscope (Zeiss Axiovert 200M, Carl Zeiss Toronto ON, Canada) and representative images were captured using a Leica DC300 digital camera and software (Leica Microsystems Canada Inc., Vaughan ON, Canada). Images shown are representative of results obtained with 3 different AD brains.

### **Central nervous system exposure**

Aged APP/PS1 (APP<sup>swe</sup>/PSEN1<sup>dE9</sup>) mice and wild type (WT) littermates (54-71 weeks old) were injected intraperitoneally (i.p.) with 30 mg/kg hu 301-17, aducanumab or vehicle (PBS) as a negative control. Plasma was collected prior to treatment and on days 1, 7, 14 and 21 for assessment of circulating levels of human IgG. Animals were sacrificed immediately after plasma collection and PBS perfusion. Brains were then collected and flash frozen. Brains and plasma were stored at -80C until use. Brain homogenates (10% w/v) were generated in RIPA buffer with an Omni tissue homogenizer (Omni International, Inc. Kennesaw, GA, USA), at half power for 30 sec, three times. Homogenates were then sonicated for 15 sec at half power, followed by clearance of debris by centrifugation (2,000xg). Levels of human IgG in plasma (1:10,000 dilution) and brain homogenates (1:5 dilution) from individual mice were measured using the Human IgG Immunotek ELISA (Zeptomatrix, Buffalo NY, USA) according to manufacturer's instructions. Results are expressed as mean µg/ml (plasma) or ng/g (brain) ±SEM

### **Results**

#### **Comparison of humanized 301-17 binding profile to other Aβ-directed antibodies**

[00340] The IgG4 isotype (S241P hinge mutation) humanized antibody VH2Vκ5 was compared to other antibodies. The humanized antibody retained selective binding for synthetic AβO vs Aβ monomers as assessed by SPR as well as lack of plaque binding by immunohistochemistry on frozen AD brain sections. As shown in Fig. 3, the Aβ-directed antibodies bapineuzumab and aducanumab, known to bind fibrils, showed robust staining of parenchymal Aβ plaque and vascular deposits while neither the parent monoclonal (mu 301-17) nor the humanized 301-17 showed any staining above background. Similar results were obtained with brain sections from aged transgenic APP/PS1 transgenic mice). Binding to plaque and vascular deposits in AD clinical trials has been associated with the induction of amyloid-related imaging abnormalities due to edema (ARIA-E) and microhemorrhages (ARIA-H).

Binding of hu301-17 to low molecular weight toxic Aβ oligomer-enriched soluble AD brain extracts

5 [00341] Examination of soluble A $\beta$  species in AD brain extracts by several investigators has indicated that the neurotoxic activity resides primarily in the low molecular weight (LMW) fraction of A $\beta$ O (dimers, trimers, tetramers, dodecamers) while high molecular weight (HMW) aggregates are largely inert though they reportedly can dissociate into LMW species. Therefore, size exclusion chromatography (SEC) of pooled soluble extracts from AD brains was performed. SEC fractionation  
10 of soluble AD brain extract gave rise to a highly reproducible pattern with protein peaks in the MW regions expected to contain LMW A $\beta$ O (Fig 4a). Fractions corresponding to ~8 –70 kDa were pooled into a LMW fraction expected to contain A $\beta$ O in the dimer to dodecamer range and excluding monomers. Fractions corresponding to >70-700 kDa were pooled into a HMW fraction. Measurements of total A $\beta$ 38, 40, 42 (MSD assay) showed all 3 species to be present in both the LMW and HMW  
15 fractions with A $\beta$ 42 representing the major aggregated A $\beta$  species (A4 assay).

[00342] Binding of immobilized hu301-17 and other A $\beta$ -directed antibodies to the LMW and HMW fractions of soluble AD brain extract was assessed by SPR. Representative results from several separate assays utilizing different pooled extracts derived from multiple AD brains are shown in Fig. 4b. The hu301-17 antibody consistently showed high and preferential binding to the LMW fraction. By  
20 comparison, aducanumab and bapineuzumab showed lower and indiscriminate binding of the LMW vs HMW fraction.

[00343] A $\beta$  protein and A $\beta$  aggregates were determined to be present in the LMW fraction but to rule out the possibility that hu301-17 may have been binding to other protein(s) also present in this fraction, a sandwich SPR assay was conducted whereby the material captured by immobilized hu301-  
25 17 was subsequently exposed to a detector antibody. Aducanumab was chosen as the detector antibody as it is known to be specific for A $\beta$  and was expected to bind/detect material captured by immobilized aducanumab, thereby acting as a positive control. As illustrated in Fig. 4c, aducanumab detection in the sandwich assay did produce a signal against material captured by either hu301-17 or aducanumab, thereby confirming the binding of hu 301-17 to A $\beta$ O in the LMW fraction. The material  
30 giving rise to the low signal observed after capture with control human IgG in this assay was not detected by aducanumab consistent with a low degree of non-specific background binding.

[00344] The specific nature of hu301-17 binding was further demonstrated in an SPR assay showing that pre-exposure of immobilized hu301-17 to its cognate cyclic peptide epitope completely prevented subsequent binding to the LMW fraction (Fig. 4d). By comparison, pre-exposure of  
35 immobilized aducanumab to the cyclic peptide epitope had no appreciable impact on its ability to subsequently bind the LMW fraction since aducanumab recognizes a different A $\beta$  epitope. Taken together, these results suggest that hu301-17, in addition to being selective for A $\beta$ O vs monomers and plaque, also exhibits superior targeting of the LMW toxic oligomer-enriched fraction of AD brain extract compared to other A $\beta$ -directed antibodies.

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**CNS exposure to hu301-17 delivered systemically**

5 **[00345]** The ability of hu301-17 to cross the blood brain barrier (BBB) and enter the central nervous system (CNS) from the periphery was assessed and compared to that of aducanumab in aged wild type mice (15-17 months old). Mice were dosed with a single intraperitoneal (i.p.) injection of 30 mg/kg antibody and levels of human IgG present in the plasma and perfused brains were measured 24 hr later by ELISA. As shown in Fig 5, equivalent amounts of hu301-17 and aducanumab were detected  
10 in plasma and brain (Fig. 5a) demonstrating a comparable degree of CNS penetrance (Fig. 5b) in the range of ~0.3% as previously reported for aducanumab [18]. As expected, no human IgG was detected in mice injected with PBS alone as a negative control (Fig 5a).

**[00346]** Additional kinetic assessment of hu301-17 was conducted in aged (13-17 months old) transgenic APP/PS1 mice and wild type littermates. Plasma and brain levels of human IgG were  
15 measured on days 1, 7, 14 and 21 after i.p. administration of 30 mg/kg. In spite of declining plasma levels over time, detectable levels of hu301-17 in the brain were still measurable out to day 21 (Fig 6a,b). Interestingly, APP/PS1 mice showed a trend for greater relative retention of hu301-17 in CNS vs plasma over time, consistent with engagement of target A $\beta$ O present in the brain of transgenic mice but not in the wild type littermates (Fig 6c). These results indicate that the CNS penetrance and  
20 pharmacokinetic properties of hu301-17 are comparable to those of other monoclonal antibodies against A $\beta$  targets.

**[00347]** 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  
25 equivalent arrangements included within the spirit and scope of the appended claims.

**[00348]** 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  
30 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 entirety.

**[00349]** 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. A chimeric or humanized antibody comprising a sequence as set forth in Table 4B or a sequence with at least 50% sequence identity thereto wherein the CDR amino acid sequences are as set forth in SEQ ID Nos: 74-79.
2. The chimeric or humanized antibody of claim 1, wherein the chimeric or humanized antibody comprises a heavy chain variable region comprising: i) an amino acid sequence as set forth in any one of SEQ ID NO: 42, 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: 42, 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).
3. The chimeric or humanized antibody of claim 1 or 2, wherein the antibody comprises a light chain variable region comprising i) an amino acid sequence as set forth any one of SEQ ID NO: 56, 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: 56, 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).
4. The chimeric or humanized antibody of any one of claims 1 to 3, 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: 41, 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: 55, 57, 59, 61, 63, 65 and 67 or a codon degenerate or optimized version thereof.
5. The chimeric or humanized antibody of any one of claims 1 to 4, wherein the antibody comprises SEQ ID NO: 42 and 56; 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).
6. The chimeric or humanized antibody of claim 5, wherein the humanized antibody has a heavy chain variable sequence and a light chain variable sequence as shown in Table 4B.

7. The chimeric or humanized antibody of any one of claim 1 to 6, wherein the antibody has a KD of at least or about  $1 \times 10^{-10}$ , at least or about  $8 \times 10^{-11}$ , at least or about  $6 \times 10^{-11}$ , at least or about  $4 \times 10^{-11}$  or at least or about  $2 \times 10^{-11}$  for a cyclic peptide with sequence of SEQ ID NO: 12.
8. The humanized antibody of any one of claims 1 to 6, wherein the antibody comprises the sequences of VH2 Vk5.
9. The antibody of any one of claims 1 to 8, wherein the antibody is an antibody binding fragment selected from Fab, Fab', F(ab')<sub>2</sub>, scFv, dsFv, ds-scFv, dimers, nanobodies, minibodies, diabodies, and multimers thereof.
10. The chimeric or humanized antibody claim 9, wherein the antibody binding fragment is a Fab fragment, optionally comprising any of the variable regions in Table 4B.
11. The humanized antibody of claim 10, wherein the Fab fragment comprises VH2 Vk5.
12. The chimeric or humanized antibody of any one of claims 1 to 11, comprising the CH1 and/or CL sequence or a part thereof of IgG4, preferably wherein the CH1 and/or CL sequence comprises SEQ ID NO: 70 or 72 or a part thereof, or a conservative variant thereof or a sequence with at least 50%, 60%, 70%, 89%, 90% or 95% sequence identity to SEQ ID NO: 70 and/or 72.
13. The chimeric or humanized antibody of any one of claims 1 to 8, wherein the antibody comprises SEQ ID NO: 70 and/or 72, and/or CH1 and CH2 of SEQ ID NO: 70 or a conservative variant of any of the foregoing or a sequence with at least 50%, 60%, 70%, 89%, 90% or 95% sequence identity to any of the foregoing.
14. The chimeric or humanized antibody of any one of claims 1 to 13 for inhibiting A-beta oligomer propagation in a subject.
15. The humanized antibody of any one of claims 1 to 13 for treating AD and/or other A-beta amyloid related diseases.
16. The antibody of any one of claims 1 to 15, wherein the antibody is a single chain antibody.
17. An immunoconjugate comprising the antibody of any one of claims 1 to 16 and a detectable label or cytotoxic agent.
18. The immunoconjugate of claim 17, wherein the detectable label comprises a positron emitting radionuclide, optionally for use in subject imaging such as PET imaging.
19. A composition comprising the antibody of any one of claims 1 to 16, or the immunoconjugate of claim 17 or 18, optionally with a diluent.

20. A nucleic acid molecule encoding the antibody of any one of claims 1 to 16.
21. A vector comprising the nucleic acid of claim 20.
22. A cell expressing an antibody of any one of claims 1 to 16, optionally wherein the cell is a hybridoma comprising the vector of claim 21.
23. A kit comprising the antibody of any one of claims 1 to 16, the nucleic acid molecule of claim 20, the vector of claim 21 or the cell of claim 22.
24. 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 and producing the chimeric antibody or humanized antibody optionally in a human IgG4 framework.
25. A method of determining if a biological sample comprises A-beta, the method comprising:
- contacting the biological sample with an antibody of any one of claims 1 to 16 or the immunoconjugate of claim 17 or 18; and
  - detecting the presence of any antibody complex.
26. The method of claim 25 for determining if the biological sample contains A-beta oligomer the method comprising:
- contacting the sample with the antibody of any one of claims 1 to 16 or the immunoconjugate of claim 17 or 18 that is specific and/or selective for A-beta oligomers under conditions permissive for forming an antibody: A-beta oligomer complex; and
  - detecting the presence of any complex;
- wherein the presence of detectable complex is indicative that the sample may contain A-beta oligomer.
27. The method of claim 26, wherein the amount of complex is measured.
28. The method of any one of claims 25 to 27, wherein the sample comprises brain tissue or an extract thereof, whole blood, plasma, serum and/or CSF.
29. The method of any one of claims 25 to 28, wherein the sample is compared to a control, optionally a previous sample.
30. 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 17 or 18 wherein the antibody is conjugated to a detectable label; and detecting the label, optionally quantitatively detecting the label.

31. The method of claim 30, wherein the label is a positron emitting radionuclide.

32. 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 18, to inhibit A-beta aggregation and/or oligomer propagation.

33. 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 1-18, 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.

34. The method of claim 33, 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.

35. The method of claim any one of claims 32 to 34, wherein the antibody, immunoconjugate, composition or nucleic acid or vector is administered directly to the brain or other portion of the CNS.

36. The method of any one of claims 32 to 35, wherein the composition is a pharmaceutical composition comprising the antibody or immunoconjugate in admixture with a pharmaceutically acceptable, diluent or carrier.

Fig. 1

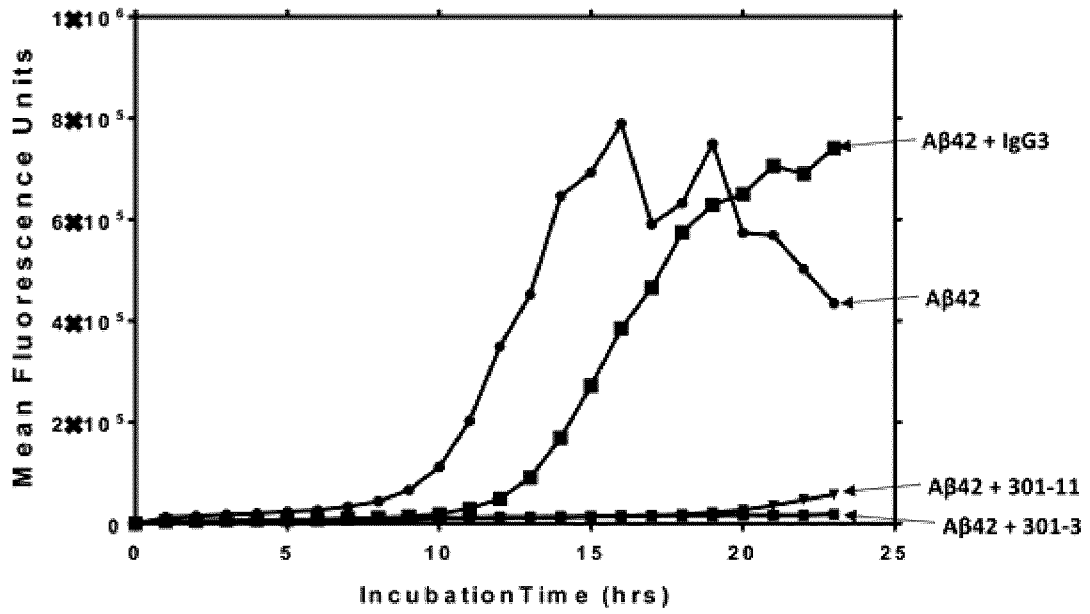
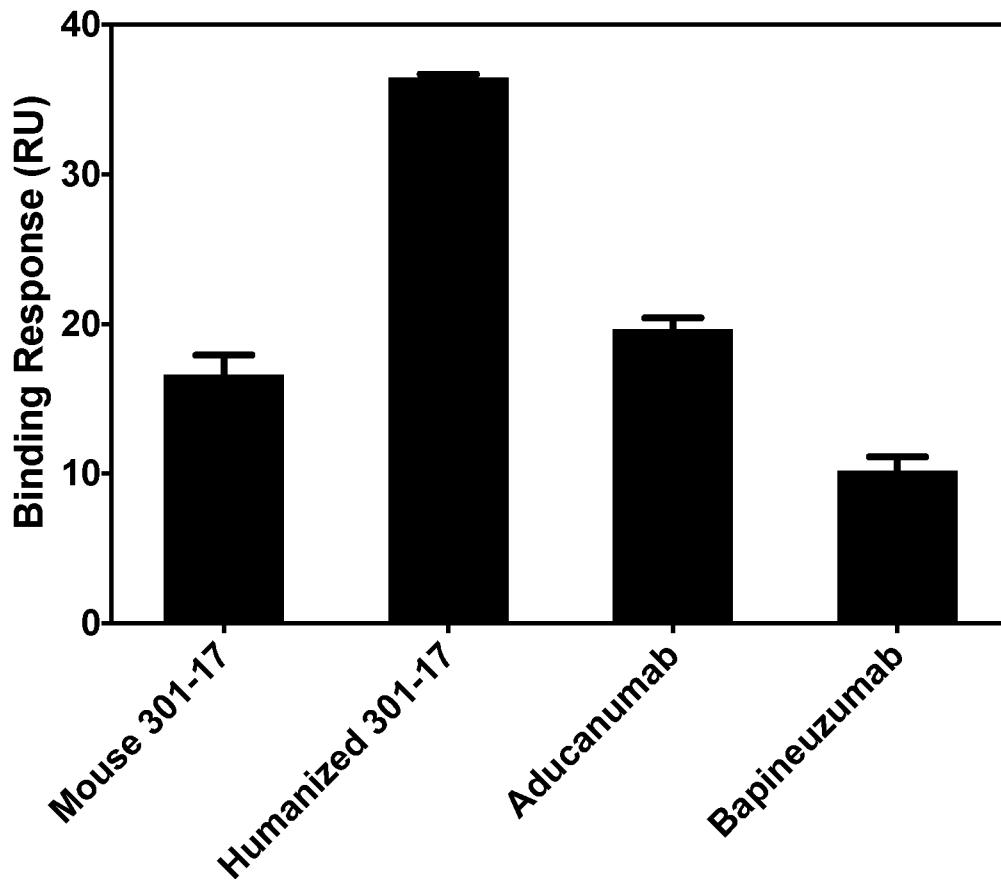
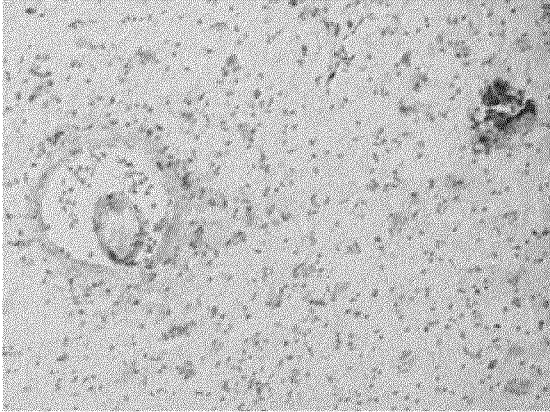


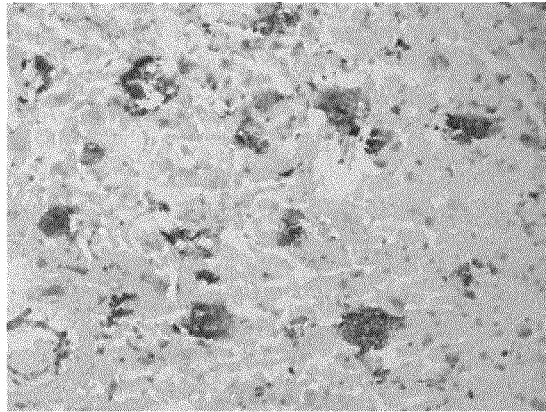
Fig. 2



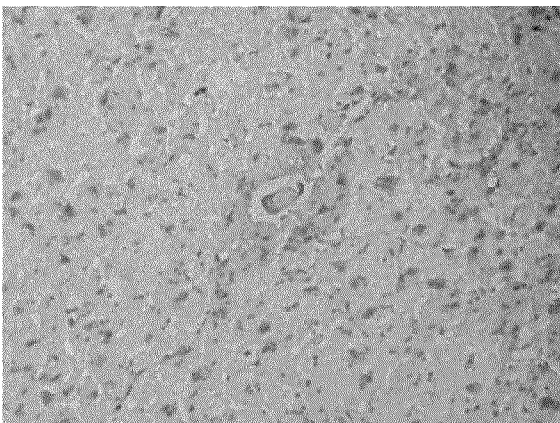
**Fig. 3A**  
**Aducanumab**



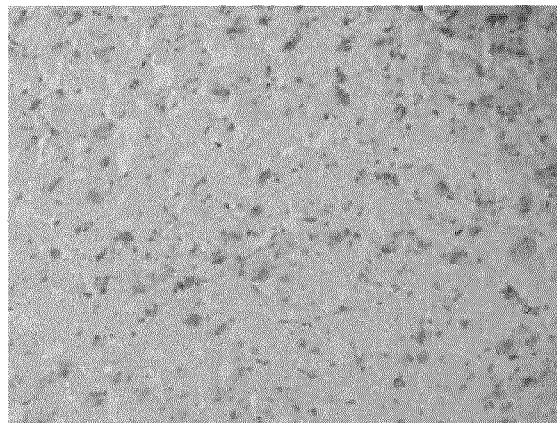
**Fig. 3B**  
**Bapineuzumab**



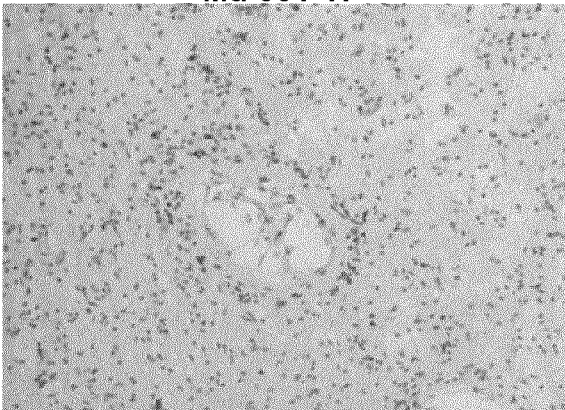
**Fig. 3C**  
**IgG1 isotype control**



**Fig. 3D**  
**Humanized 301-17**



**Fig. 3E**  
**Mu 301-17**



**Fig. 3F**  
**IgG4 isotype control**

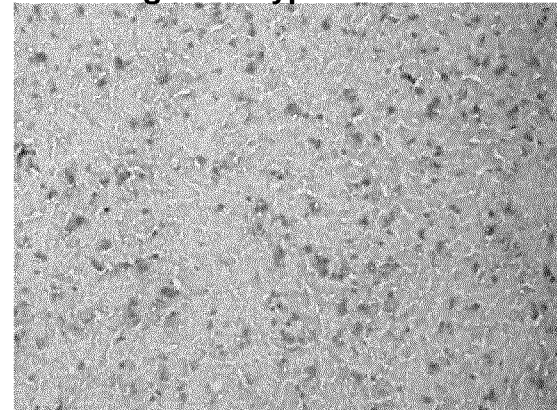


Fig. 4a

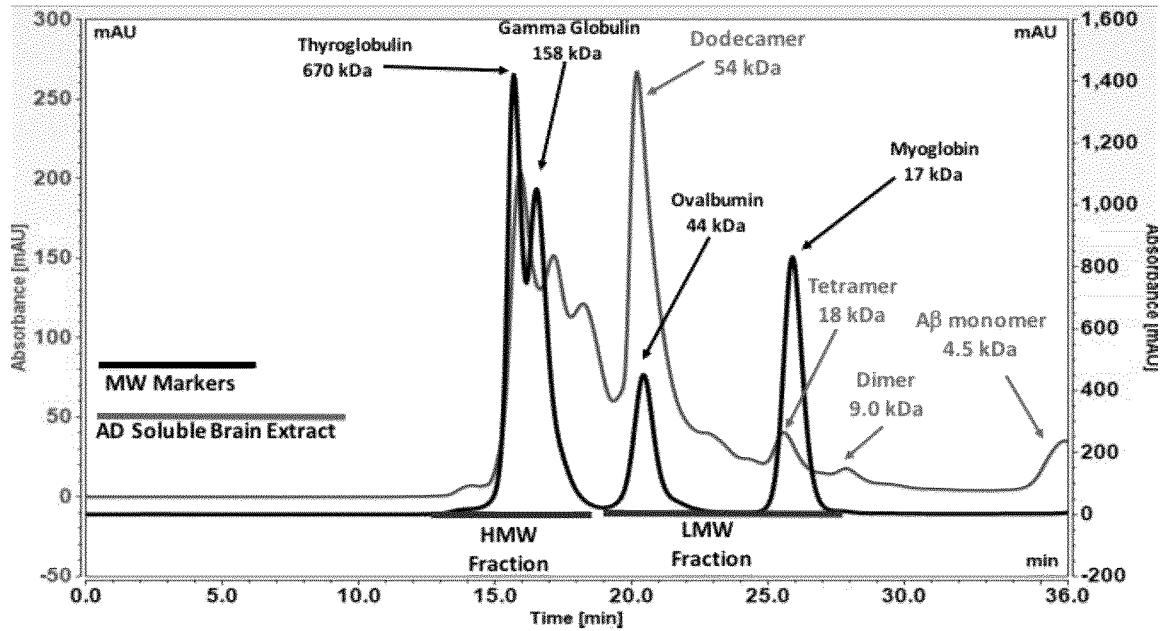


Fig. 4b

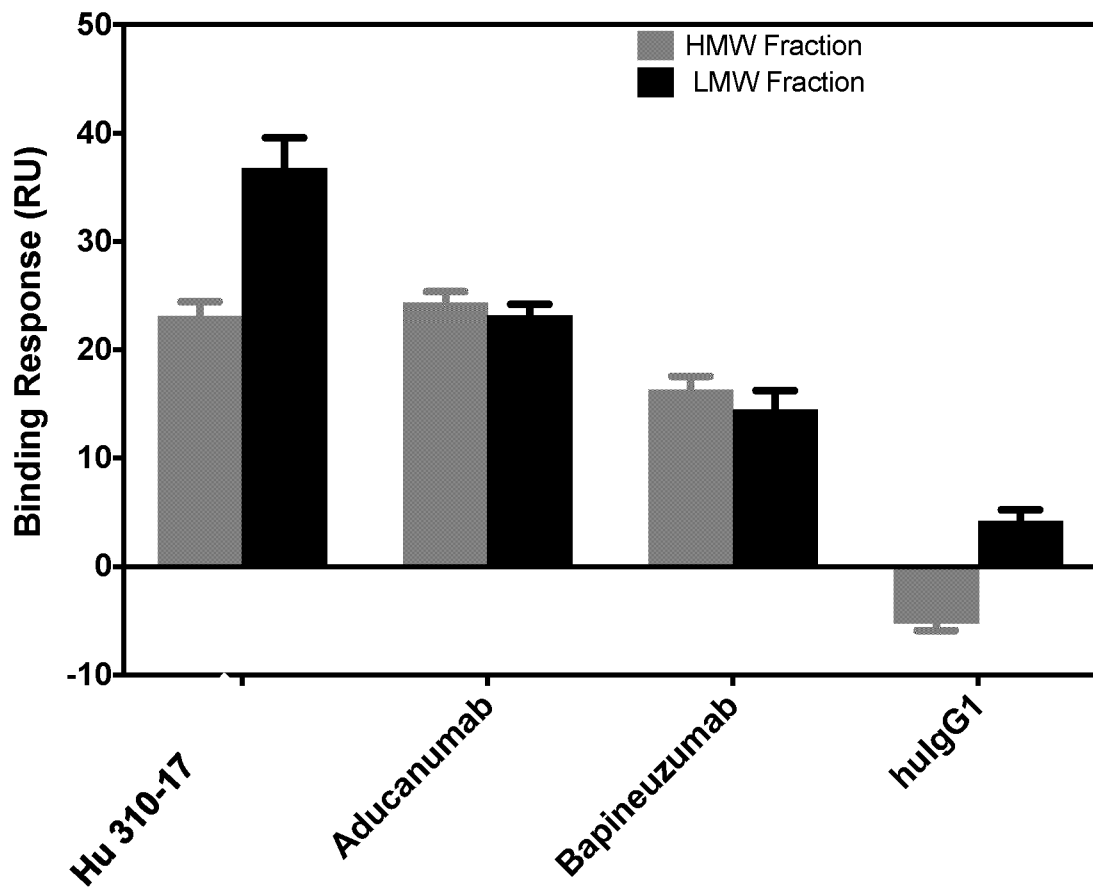


Fig. 4c

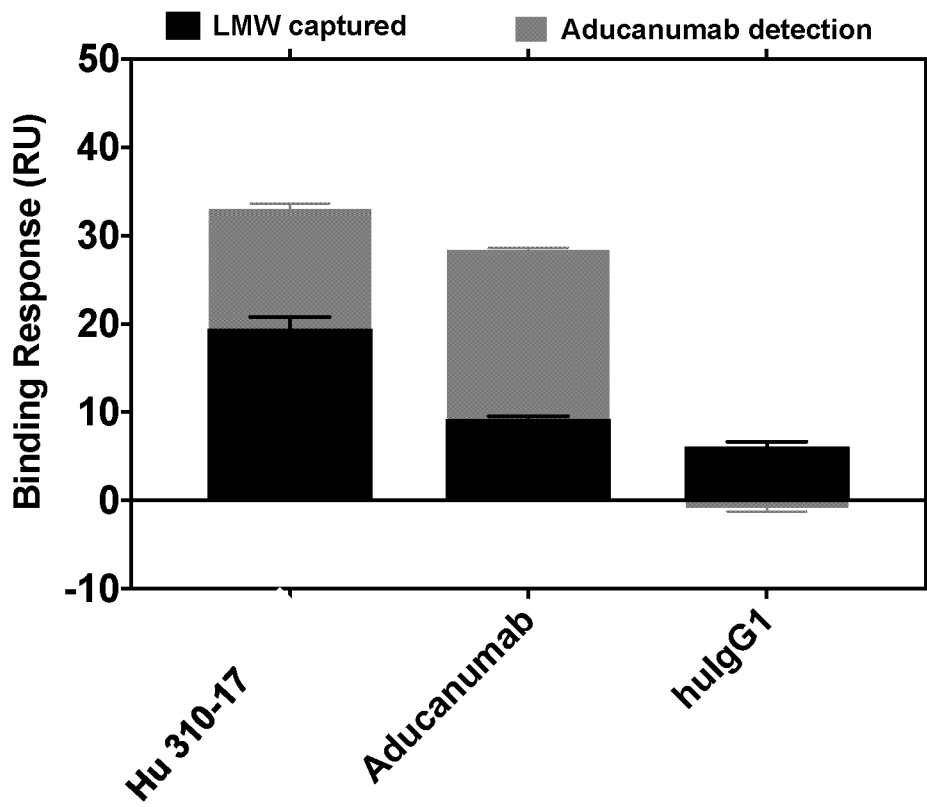
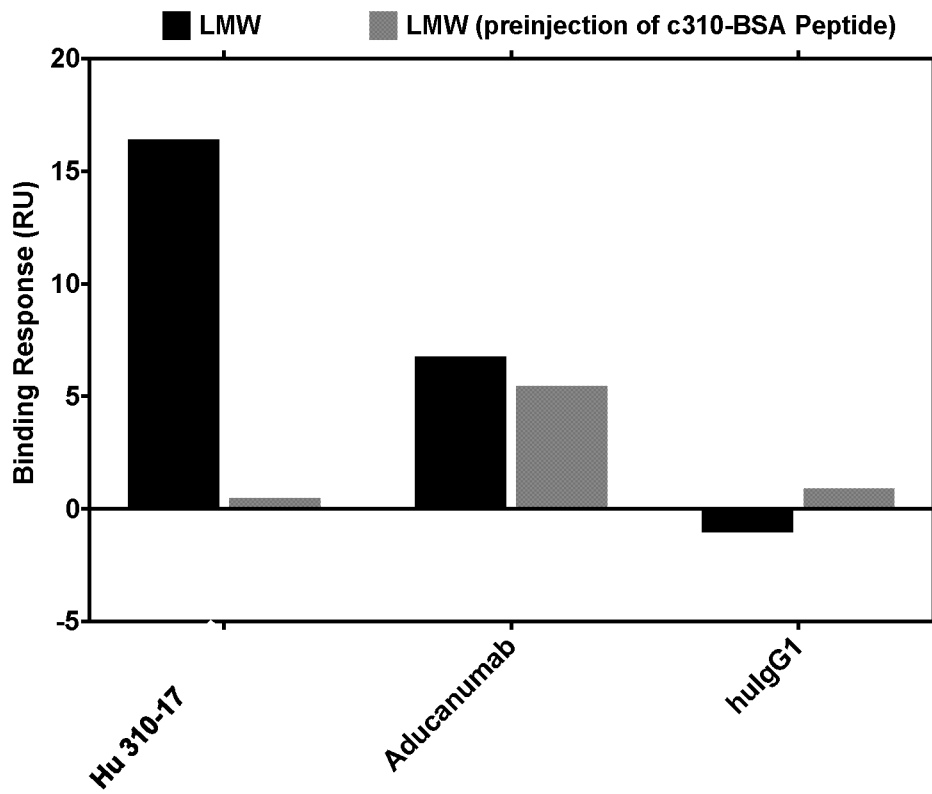


Fig. 4d



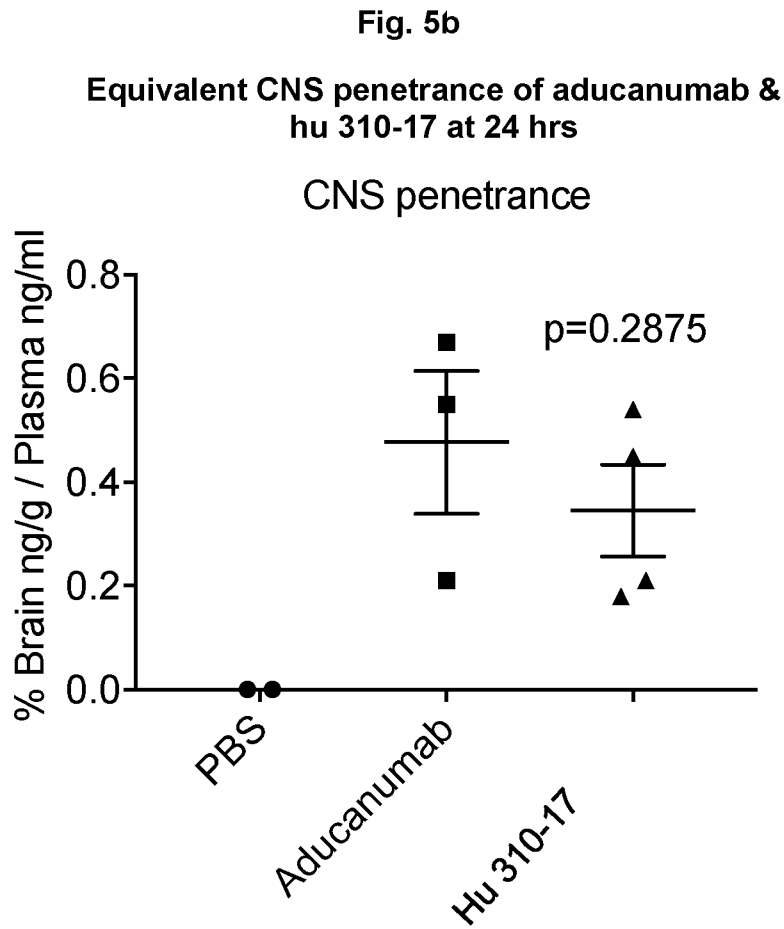
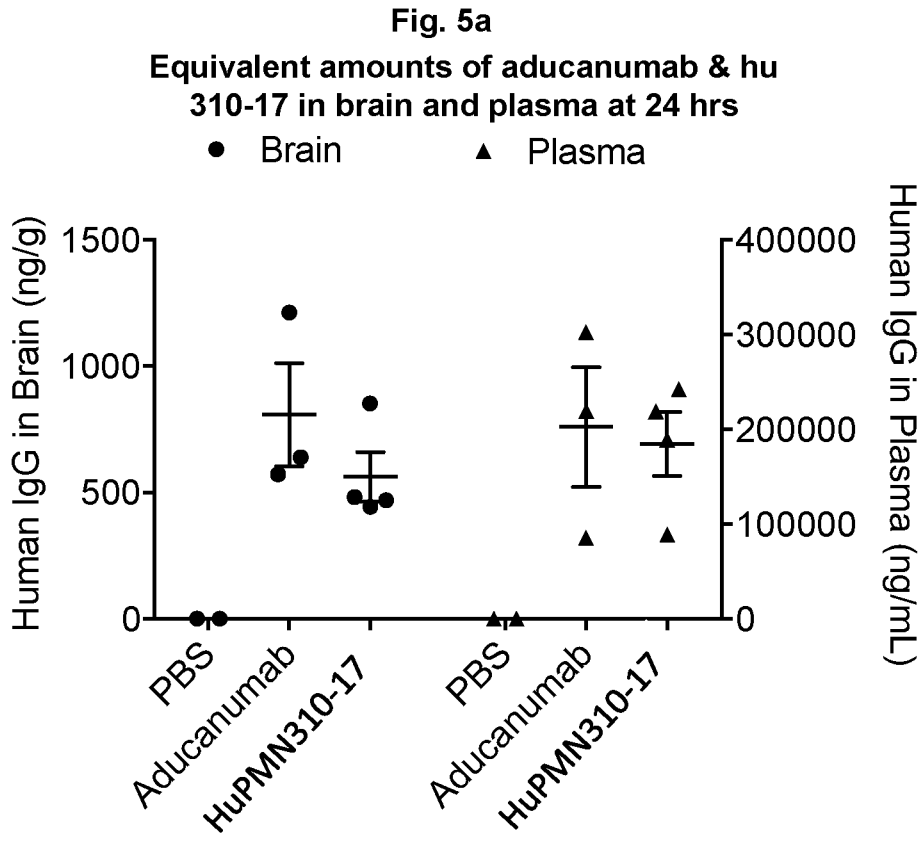


Fig. 5c

Non-Tg

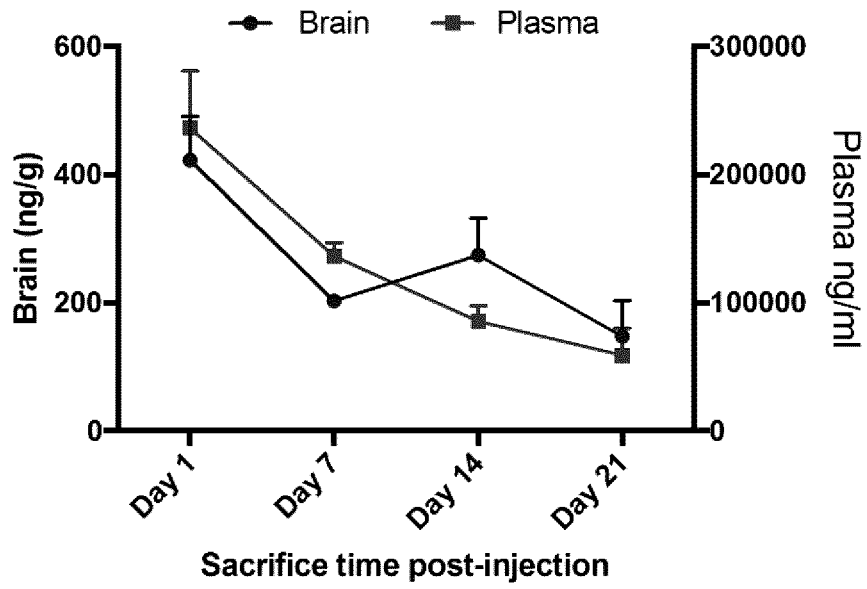


Fig. 5d

APP/PS1

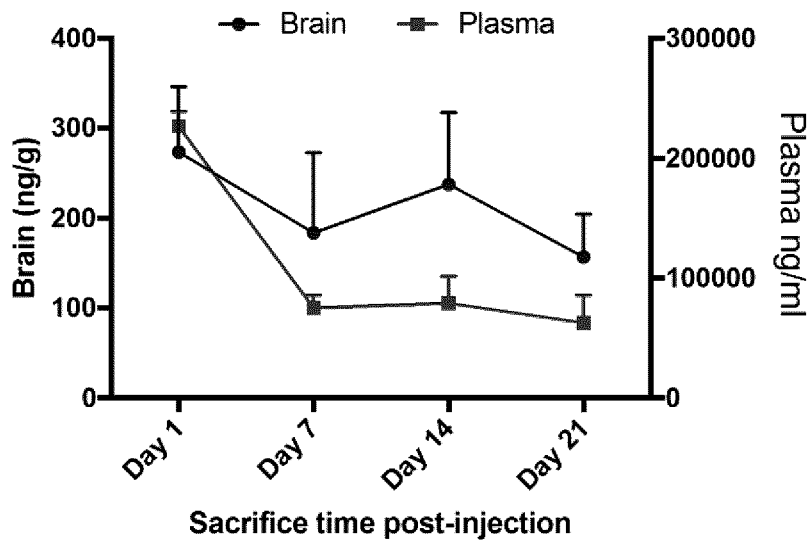
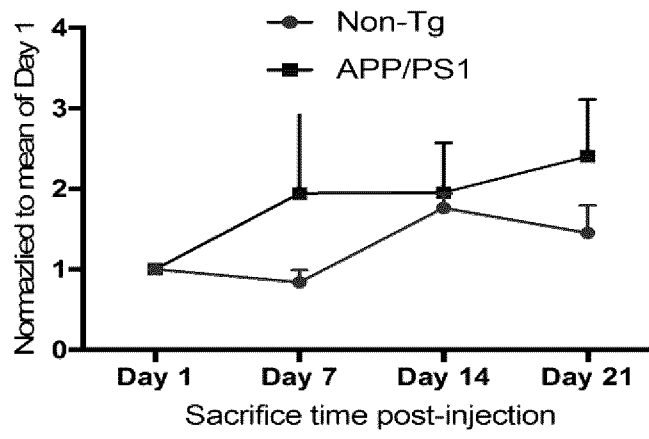


Fig. 5e

Brain/plasma ratio



## INTERNATIONAL SEARCH REPORT

International application No.

**PCT/CA2018/050875**

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC: <i>C07K 16/46</i> (2006.01), <i>A61K 39/395</i> (2006.01), <i>A61K 47/68</i> (2017.01), <i>A61K 49/00</i> (2006.01), <i>A61K 51/10</i> (2006.01), <i>C07K 16/18</i> (2006.01), <i>C12N 15/13</i> (2006.01), <i>C12N 5/16</i> (2006.01), <i>G01N 33/53</i> (2006.01) According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC: ALL 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) Questel, GQ-Pat, Genbank, Geneseq, Protein Data Bank, Genpept: amyloid, SEQ ID NOs: 42, 44, 46, 48, 50, 52, 54, 56, 58, 60, 62, 64, 66, 68, 70, 72 and 74-79		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO2017079833 (CASHMAN, N.R. et al.) 18 May 2017 (18-05-2017)	1-7, 9, 10, 12 and 14-36
Y		13
Y	WO2017025918 (BILIC, S. et al.) 16 February 2017 (16-02-2017)	13
P, X	US20180125920 (CASHMAN, N.R. et al.) 10 May 2018 (10-05-2018)	1-36
<input type="checkbox"/> Further documents are listed in the continuation of Box C.		
<input checked="" type="checkbox"/> See patent family annex.		
* "A" "E" "L" "O" "P"	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier application or patent but published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	"I" 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 24 September 2018 (24-09-2018)		Date of mailing of the international search report 25 September 2018 (25-09-2018)
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  Rena Oulton (819) 639-6855

**INTERNATIONAL SEARCH REPORT**  
Information on patent family members

International application No.  
**PCT/CA2018/050875**

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Continued on extra page.

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专利名称(译)	抗体VS淀粉样蛋白		
公开(公告)号	<a href="#">EP3574020A1</a>	公开(公告)日	2019-12-04
申请号	EP2018835520	申请日	2018-07-18
[标]申请(专利权)人(译)	英属哥伦比亚大学 PROMIS NEUROSCI		
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发明人	CASHMAN, NEIL R. PLOTKIN, STEVEN S. KAPLAN, JOHANNE SILVERMAN, JUDITH MAXWELL GIBBS, EBRIMA		
IPC分类号	C07K16/46 A61K39/395 A61K47/68 A61K49/00 A61K51/10 C07K16/18 C12N15/13 C12N5/16 G01N33/53		
CPC分类号	A61K2039/505 A61P25/28 C07K16/18 C07K2317/24 C07K2317/92 G01N33/6896 G01N2333/4709		
优先权	PCT/CA2017/050866 2017-07-18 WO 15/808842 2017-11-09 US 62/622126 2018-01-25 US		
其他公开文献	EP3574020A4		
外部链接	<a href="#">Espacenet</a>		

#### 摘要(译)

本公开涉及结合A-β寡聚体的抗体以及制备和使用所述抗体的方法。还提供了嵌合或人源化抗体，包括表2中具有CDR的抗体和/或具有表4B中列出的序列的抗体或具有至少50%序列同一性的序列，其中CDR氨基酸序列如下所述。在SEQ ID Nos : 74-79中。还提供了其方法和用途以及包含所述抗体的试剂盒。