the samples were centrifuged for 10 min at 10000 g. The supernatants were discarded, and the residues were mixed with 20 µL 20 mM NaH_2PO_4, 140 mM NaCl, 0.025% Tween 20, pH 7.4, then with 20 µL of SDS-PAGE sample buffer. The samples were applied to a 4 – 20% Tris/glycine gel for electrophoresis.

Parameters for SDS-PAGE:

SDS sample buffer: 0.3 g SDS
4 mL 1M Tris/HCl pH 6.8
8 mL glycerine
1 mL 1% bromphenol blue in ethanol
Fill with H_2O ad 50 mL

4-20% Tris/Glycine Gel: (Invitrogen Cat.no.: EC6025BOX)

Electrophoresis buffer: 7.5 g Tris
36 g Glycine
2.5 g SDS
Fill with H_2O ad 2.5 L

The gel is run at a constant current of 20 mA.
Staining of the gels: Coomassie Blue R250

Results are shown in Figure 3.

Semiquantitative analysis of different anti-Aβ antibodies and their discrimination of Aβ(1-42) fibrils:

Positions of antibodies, Aβ(1-42) fibrils antibody heavy chain, antibody light chain and Aβ(1-42) monomers are marked at the edge of the gel. Due to their size, Aβ(1-42) fibrils cannot enter the SDS-PAGE gel and can be seen in the gel slot.

1. Marker

2. Aβ(1-42) fibril preparation; control
3. Aβ(1-42) fibril preparation; + mAb 6E10; 20h 37°C; supernatant
4. Aβ(1-42) fibril preparation; + mAb 6E10; 20h 37°C; pellet
5. Aβ(1-42) fibril preparation; + mAb 3C5; 20h 37°C; supernatant
6. Aβ(1-42) fibril preparation; + mAb 3C5; 20h 37°C; pellet
7. Aβ(1-42) fibril preparation; + mAb 10F4; 20h 37°C; supernatant
8. Aβ(1-42) fibril preparation; + mAb 10F4; 20h 37°C; pellet

The relative binding to fibril type Aβ was evaluated from SDS-PAGE analysis by measuring the Optical Density (OD) values from the Heavy Chain of the antibodies in the fibril bound (pellet-fraction) and the supernatant fractions after centrifugation. Antibodies that have bound to the Aβ fibrils should be co-pelleted with the Aβ-fibrils and therefore are found in the pellet fraction whereas non-Aβ-fibril bound (free) antibodies are found in the supernatant. The percentage of antibody bound to Aβ-fibrils was calculated according to the following formula:

Percent antibody bound to Aβ-fibrils =
$OD_{\text{fibril fraction}}$ x100%/($OD_{\text{fibril fraction}}$ + $OD_{\text{supernatant fraction}}$).

Results are shown in Figure 3. In contrast to the commercially available antibody 6E10 (Signet Cat. no.: 9320) which recognizes and binds to a linear Aβ-epitope between AA1-17, the Aβ globulomer antibodies 3C5 and 10F4 bind to Aβ(1-42)-fibrils with a lower affinity in a co-pelleting

experiment. This is evidenced by the fact that the 3C5 and 10F4 antibodies, after an incubation with Aβ(1-42) fibrils, remain mainly after a pelleting step in the supernatant and are not co-pelleted due to being bound to the Aβ(1-42) fibrils.

In the Alzheimer's disease brain, the Aβ fibrils are a major component of the total Aβ peptide pool. By attacking these fibrils by anti Aβ-antibodies, the risk of negative side effects is elevated due to a liberation of high amounts of Aβ which subsequently may increase the risk of microhaemorrhages. An increased risk for microhemorrhages was observed in an active immunization approach with fibrillar aggregates of the Aβ peptide (Bennett and Holtzman, 2005, Neurology, 64, 10-12; Orgogozo J, Neurology, 2003, 61,46-54; Schenk et al., 2004, Curr Opin Immunol, 16, 599-606).

EXAMPLE VI

IN SITU ANALYSIS OF THE SPECIFIC REACTION OF ANTIBODIES 10F4 AND 3C5 TO FIBRILLAR ABETA PEPTIDE IN THE FORM OF AMYLOID PLAQUES AND AMYLOID IN MENINGEAL VESSELS IN OLD APP TRANSGENIC MICE AND ALZHEIMER'S DISEASE PATIENTS

Antibodies 10F4 and 3C5 show reduced staining to fibrillar Aβ peptide deposits suggesting that their therapeutic effect is mediated by binding to soluble globulomeric forms rather than fibrillar deposited forms of Aβ peptide. Since antibody binding to fibrillar Aβ peptide can lead to fast dissolution of aggregates and a subsequent increase of soluble Aβ concentration, which in turn is thought to be neurotoxic and could lead to microhemorrhages, an antibody therapy that effects the soluble globulomer rather than the monomer is preferred.

Methods:

For these experiments, several brain material samples were used: cortical tissue from 2 AD patients (RZ16 and RZ 55)and cortical tissue from 19 month old Tg2576 mice (APPSWE # 001349,Taconic, Hudson, NY, USA) or 12 month old APP/L mice (ReMYND, Leuven, Belgium).
The mice overexpress human APP with a familial Alzheimer's disease mutation and form β-amyloid deposits in the brain parenchyma at about 11 months of age and β-amyloid deposits in larger cerebral vessels at about 18 months of age. The animals were deeply anaesthetized and transcardially perfused with 0.1 M phosphate-buffered saline (PBS) to flush the blood. Then, the brain was removed from the cranium and divided longitudinally. One hemisphere of the brain was shock-frozen and the other fixated by immersion into 4% paraformaldehyde. The immersion-fixated hemisphere was cryoprotected by soaking in 30% sucrose in PBS and mounted on a freezing microtome. The entire forebrain was cut into 40 µm transverse sections which were collected in PBS and used for the subsequent staining procedure.
The neocortex samples from Alzheimer's disease patients were obtained from Brain-Net, Munich, Germany as frozen tissue, immersion-fixated in 4% paraformaldehyde during thawing, and subsequently treated like the mouse tissue.

Individual sections were stained with Congo Red using the following protocol:

Material:

- Amyloid dye Congo Red kit (Sigma-Aldrich; HT-60), consisting of alcoholic NaCl solution, NaOH solution and Congo Red solution
- staining cuvettes
- microscope slides SuperfrostPlus and coverslips

- Ethanol, Xylol, embedding medium

Reagents:

- NaOH diluted 1:100 with NaCl solution yields alkaline saline
- alkaline saline diluted 1:100 with Congo Red solution yields alkaline Congo Red solution (prepare no more than 15 min before use, filtrate)
- mount sections on slide and allow them to dry
- incubate slide in staining cuvette, first for 30-40 minutes in alkaline saline, then for 30-40 minutes in alkaline Congo Red solution
- rinse three times with fresh ethanol and embed over xylol

Staining was first photographed using a Zeiss Axioplan microscope (Zeiss, Jena, Germany) and evaluated qualitatively. Red colour indicated amyloid deposits both in the form of plaques and in larger meningeal vessels. Later on, evaluation of antibody staining focused on these structures.

Staining was performed by incubating the sections with a solution containing 0.07 - 0.7 µg/ml of the respective antibody in accordance with the following protocol:

Materials:

- TBST washing solution (Tris Buffered Saline with Tween 20; 10x concentrate; DakoCytomation S3306, DAKO, Hamburg, Germany) 1:10 in Aqua bidest.)
- 0.3% H_2O_2 in methanol
- donkey serum (Serotec, Düsseldorf, Germany), 5% in TBST, as blocking serum
- monoclonal mouse-anti-globulomer antibodies diluted at given concentrations in TBST

- secondary antibody: biotinylated donkey-anti-mouse antibody (Jackson Immuno / Dianova, Hamburg, Germany; 715-065-150; diluted 1:500 in TBST)
- StreptABComplex (DakoCytomation K 0377, DAKO, Hamburg, Germany)
- Peroxidase Substrate Kit diaminobenzidine (=DAB; SK-4100; Vector Laboratories, Burlingame, CA, USA)
- SuperFrost Plus microscope slides and coverslips
- xylol free embedding medium (Medite, Burgdorf, Germany; X-tra Kitt)

Procedure:

- transfer floating sections into ice-cold 0.3% H_2O_2 and incubate for 30 min
- wash for 5 min in TBST buffer
- incubate with donkey serum/TBST for 20 minutes
- incubate with primary antibody for 24 hours at room temperature
- wash in TBST buffer for 5 minutes
- incubate with blocking serum for 20 minutes
- wash in TBST buffer for 5 minutes
- incubate with secondary antibody for 60 minutes at ambient temperature
- wash in TBST buffer for 5 minutes
- incubate with StreptABComplex for 60 minutes at ambient temperature
- wash in TBST buffer for 5 minutes
- incubate with DAB for 20 minutes
- mount the section on slides, air-dry slides, dehydrate slides with alcohol and embed slides

Besides visual inspection of sections under the microscope, amyloid staining was additionally quantified by optically excising 10 randomly selected plaques from the histological

images using the ImagePro 5.0 image analysis system and determining their average greyscale value. Optical density values (were calculated from the greyscale values by subtracting the mean background density of the stained material from the density of amyloid plaques (0% - no plaque staining above surrounding background, 100% - no transmission / maximal staining). The differences between between antibodies 6E10 / 4G8 and 6G1, 10F4 and 3C5 , respectively, were tested for statistical significance with ANOVA.

Results:

All antibody stained material described in the following proved to be congophilic amyloid deposits (Fig. 4(a)). The globulomer-preferring antibodies 10F4 and 3C5 stained parenchymal and meningeal congophilic deposits of Aβ peptide at the same concentration of 0.7 µg/mL significantly less than the antibodies 6G1 and 6E10 (Fig. 4(b,c,h)). Quantitative analysis of parenchymal amyloid plaque staining revealed binding of all antibodies to plaques (statistically significant density above control), but binding of antibody 10F4 and 3C5 was significantlyly lower than binding of the reference antibody 6E10 (raised to N-terminal sequence of Aβ) and equal or lower than reference antibody 4G8 (raised to N-terminal sequence of Aβ)(Fig. 4(d-g)).
Antibodies 10F4 and 3C5 bind less to amyloid deposits than antibodies which recognize Aβ monomer or part of the Aβ sequence. Treatment with antibodies binding to fibrillar Aβ peptide can lead to fast dissolution of amyloid plaques in brain tissue and a subsequent increase of soluble Aβ concentration, which in turn is thought to be neurotoxic and could lead to microhemorrhages, and/or a fast dissolution of vascular amyloid, which also could lead to microhemorrhages.

Therefore, an antibody therapy that effects the soluble globulomer rather than the monomer is preferred.

EXAMPLE VII

ENDOGENOUS Aβ(1-42) AND Aβ(1-40) LEVELS IN CSF OF AD PATIENTS AFTER IMMUNOPRECIPITATION WITH ANTI-Aβ GLOBULOMER ANTIBODIES 10F4 AND 3C5

Immunoprecipitation (IP) of Aβ-species from AD-brain CSF with Dynabeads M-280 Sheep anti-Mouse IgG

The following mAbs were immobilized to Dynabeads M-280 Sheep anti-Mouse IgG:

- mAb 6E10 (Signet Inc.; Cat. no.: 9320)
- mAb 3C5
- mAb 10F4
- mAb 8F5

Dynabeads M-280 Sheep anti-Mouse IgG:

Sheep anti-Mouse IgG (Invitrogen Inc., Cat. no.: 112.02) is covalently bound to magnetic beads (Dynabeads).

Activation of Dynabeads with monoclonal mouse antibodies

- the stock-suspension of dynabeads (Dynabeads M-280 Sheep anti-Mouse IgG, Invitrogen; Prod. No. 112.02) was shaken carefully to prevent foaming
- 1 mL was aseptically removed and transfered to a 1.5 mL reaction vial
- the dynabeads were washed 3 times 5 min with 1 mL immunoprecipitation (IP)-wash buffer (IP-wash-buffer: PBS

(20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1% (w/v) BSA). During the washing procedure the supernatant was carefully removed while the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS)

- the washed dynabeads were incubated with 40 µg Aβ-antibody in 1 mL PBS, 0.1% (w/v) BSA
- the activation was carried out by overnight incubation under shaking at 4°C
- the activated dynabeads were washed 4 times 30 min (again using the MSS) with 1 mL IP-wash buffer (PBS (20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4), 0.1% (w/v) BSA)
- the activated dynabeads were resuspended with 1 mL PBS, 0.1% (w/v) BSA, 0.02% (w/v) Na-Azide; vortexed and centrifuged briefly
- the antibody activated dynabeads were stored at 4°C until further use

CSF Sample preparation:

400 µL CSF from an Alzheimer`s disease patient were added to 4 µL Complete Protease Inhibitor Cocktail (Roche Inc. Cat. no.: 1697498, 1 tablet dissolved in 1 mL water) and 0.8 µL 500 mM PMSF dissolved in methanol. After 10 min 1.6 mL 20 mM NaH_2PO_4,140 mM NaCl, 0.05% Tween 20, pH 7.4 (PBST) was added.

Immunoprecipitation of Aβ species from human AD-CSF:

250 µL aliquot of the prepared CSF sample were added to 25 µL anti-Aß-Dynabeads suspension

- Immunoprecipitation occured under stirring at 6°C for 16 hours. Subsequent washing of the beads was performed 3

times 5 min. with 1 mL PBS/0,1% (w/v) BSA and finally once 3 min. with 1 mL 10 mM Tris/HCL pH 7.5 buffer. During the washing procedure the supernatant was carefully removed while the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS)

The residual supernatant was thoroughly removed after the final washing step.

The Aβ peptides and the corresponding antibody were removed from the Dynabeads by adding 25µL sample buffer without β-Mercaptoethanol (0.36 M Bistris, 0.16 M Bicine, 1% SDS (w/v), 15% (w/v) sucrose, 0.004% (w/v) Bromphenolblue) to the Eppendorff tube and heating for 5 min at 95°C in a heating block. After cooling to room temperature the dynabeads were immobilized at the side of the reaction vial with a magnetic separator stand (MSS) and the supernatant were transferred to another Eppendorff tube (IP eluate).

Analysis of Aβ immunoprecipitates by urea-PAGE followed by Western Blot procedure:

The quantification of Aβ1-40 and Aβ1-42 species was performed by a 8 M Urea Poly-Acrylamide-Gel-Electrophoresis system and subsequent Western Blot analysis according to the procedure first described by H.W. Klafki et al., Analytical Biochemistry 237., 24-29 (1996) and later also used by J. Wiltfang et al., J. of Neurochemistry 81, 481-496, 2002. There were only two minor changes made in the experimental procedure:

1) SDS concentration in the stacking gel was adjusted to 0.25% (w/v) instead of 0.1% (w/v).
2) For the Western blot the antibody 1E8 (Senetek Drug Delivery Technologies Inc. St.Louis, MO,

USA) was replaced by Anti-Human Amyloid β (N) (82E1) Mouse IgG mAb (IBL, Cat.no.: 10323)

15 µL IP eluate aliquots of the immunoprecipitated samples were loaded onto the 8 M Urea PAGE. Electrophoresis was performed at 100 V (15 min) and continued at 60 V. The electrophoresis was stoppep when the the running front of the blue sample loading dye was still 0.5 cm away from the end of the gel.

Western blot procedure:

Western blot analysis was performed in a Semi Dry Blotting chamber (BioRad Inc., 45min at 75mA) onto 7.5cm x 9cm Nitrocellulose 0.45µm (BioRad Inc.)

Blotting buffer : 6 g Tris; 28.1 g Glycin; 500m L Methanol; adjust to 2.5 l with water.

The Nitrocellulose blot was boiled for 10 min in PBS at 100°C. The blot was saturated by treatment with 50 mL 5 % (w/v) BSA in PBST for 1 hour at RT. After removal of the fluid phase the following washing step were performed twice with: 50 mL TTBS (25 mM Tris / HCl; 150 mM NaCl Puffer; 0.05% Tween 20; pH 7.5) for 10 min at RT and subsequently with 50 mL TBS (25 mM Tris / HCl; 150 mM NaCl buffer; pH 7.5) for 10 min at RT.

For further development the final washing buffer was discarded from the blot and 15 mL antibody I solution (0.2 µg/mL 82E1 = 1:500 in 3 % (w/v) skimmed milk powder (Lasana Inc.), in 15 mL TBS) were added for 20 hours at 6°C. Removal of buffer was followed by the three wash steps as described above. The blot was incubated with Antibody solution II (1:10000 dilution of

anti-Mouse -POD in 15 mL 3 % (w/v) skimmed milk powder in 15 mL TBS) for 1 hour at RT. Removal of buffer was followed by the three wash steps as described above.

After removal of the last washing buffer 2 mL Super Signal West Femto Maximum Sensitivity Substrat Enhancer and 2 mL Peroxide Solution was mixed. The freshly prepared solution was poored onto the blot which was preincubated in the dark for 5 min. Chemoluminescence was recorded using a VersaDoc Imaging system(BioRad).

Imaging parameters:
exposure time 180 sec.
Picture records after 30 sec., 60sec., 120 sec. and 180 sec.

The results were obtained from the picture with 180 sec. exposure time.

The anti-globulomer antibodies 10F4 and 3C5 of the present invention have a lower affinity for Aβ(1-42) peptide and Aβ(1-40) peptide in the CSF of an Alzheimer's disease patient, in comparison to the commercially available antibody 6E10 (which is, in the literature, regarded to recognize all Aβ-forms regardless of their conformation). . CSF Aβ-peptide forms undergo a high turnover rate (Bateman et al., Nature Medicine, 2006, 12(7):856-61) and are therefore unlikely the disease relavant species. Therefore, the CSF Aβ-forms should not be targeted in a passive immunization treatment strategy of Alzheimer's disease in order to reduce the risk of undesired side effects. It is noted that, in an earlier study (Barghorn et al., J Neurochem. 2005; 95(3):834-847), the anti Aβ-globulomer antibody 8F5 did not recognize and bind to Aβ-

peptide in the CSF of an Alzheimer's disease patient. This ealier study was performed using a sandwich ELISA method. In contrast, when using the immunoprecipitation and Urea PAGE method described above, the same antibody 8F5 does recognize Aβ-peptide in the CSF of an Alzheimer's disease patient (see Figure 5). Therefore, the sandwich ELISA method produced false negative results; hence, for the detection of Aβ-peptides in CSF, the immunoprecipitation and Urea PAGE methods described herein should be used.

WHAT IS CLAIMED IS:

1. An isolated antibody having a higher affinity to Aβ(1-42) globulomer than to at least one amyloid beta protein selected from the group consisting of Aß(1-42) peptide present in cerebrospinal fluid (CSF) and b) Aß(1-40) peptide present in CSF.

2. An isolated antibody having a binding affinity to Aβ(1-42) globulomer which is greater than the binding affinity to at least one amyloid beta protein selected from the group consisting of a) Aβ(1-42) monomer, b) Aβ(1-40) monomer, c) Aβ(1-42) fibril and d) soluble amyloid precursor protein-alpha (sAPPα).

3. The isolated antibody of claim 2 wherein said antibody binds with less affinity to amyloid beta protein present in non-CSF than to amyloid beta protein present in CSF.

4. The isolated antibody of claim 1 or claim 2, wherein the antibody is a monoclonal antibody.

5. The isolated antibody of claim 4, wherein the antibody is a recombinant antibody.

6. The isolated antibody of claim 1 or claim 2, wherein the antibody is human or humanized.

7. The isolated antibody of claim 1 or claim 2, wherein said antibody binds to at least one epitope as a monoclonal antibody selected from the group consisting of the monoclonal antibody 10F4 obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808 and

the monoclonal antibody 3C5 obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

8. An isolated antibody comprising SEQ ID NO:5.

9. An isolated antibody comprising SEQ ID NO:6.

10. The isolated antibody of claim 9 further comprising SEQ ID NO:5.

11. An isolated antibody comprising SEQ ID NO:7.

12. An isolated antibody comprising SEQ ID NO:8.

13. The isolated antibody of claim 12 further comprising SEQ ID NO:7.

14. The isolated antibody of claim 1 or claim 2, wherein the antibody comprises at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR3 and the amino acid sequence of the light chain CDR3 of a monoclonal antibody (10F4) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808 and b) the amino acid sequence of the heavy chain CDR3 and the amino acid sequence of the light chain CDR3 of a monoclonal antibody (3C5) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

15. The isolated antibody of claim 1 or claim 2, wherein the antibody comprises at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR2 and the amino acid sequence of the light

chain CDR2 of a monoclonal antibody (10F4) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808 and b) the amino acid sequence of the heavy chain CDR2 and the amino acid sequence of the light chain CDR2 of a monoclonal antibody (3C5) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

16. The isolated antibody of claim 1 or claim 2, wherein the antibody comprises at least one amino acid sequence selected from the group consisting of: a) the amino acid sequence of the heavy chain CDR1 and the amino acid sequence of the light chain CDR1 of a monoclonal antibody (10F4) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808 and b) the amino acid sequence of the heavy chain CDR1 and the amino acid sequence of the light chain CDR1 of a monoclonal antibody (3C5) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

17. An isolated antibody comprising at least one CDR selected from the group consisting of amino acid sequence: a) SHYAWN; b) YIDYSGSTRYLPSLKS; c) GSGYFYGMDY; d) HASQNINVWLS; e) KASNLHT; f) QQGQSYPYT; g) NYLIE; h) VINPGSGDTNYNENFKG; i) GVITTGFDY; j) RASGNIHNYLA; k) NAKTLAD and l) QHFWSSPRT.

18. A hybridoma designated by American Type Culture Collection deposit number PTA-7808.

19. A monoclonal antibody (10F4) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7808.

20. A hybridoma designated by American Type Culture

Collection deposit number PTA-7406.

21. A monoclonal antibody (3C5) obtainable from a hybridoma designated by American Type Culture Collection deposit number PTA-7406.

22. An isolated nucleic acid molecule encoding the antibody of claim 1 or claim 2.

23. The isolated nucleic acid molecule of claim 22 wherein the nucleotide sequence of said molecule comprises at least one sequence selected from the group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

24. A vector comprising said isolated nucleic acid molecule of claim 23.

25. A host cell comprising said vector of claim 24.

26. A method of producing an antibody, comprising culturing said host cell of claim 25 in a culture medium for a time and under conditions suitable for production of said antibody.

27. An isolated antibody produced by said method of claim 26.

28. A composition comprising said antibody of claim 1, said antibody of claim 2 or a combination thereof.

29. The composition of claim 28, wherein the composition is a pharmaceutical composition and further comprises a pharmaceutical acceptable carrier.

30. A monoclonal antibody comprising an amino acid sequence encoded by at least one nucleotide sequence selected from the

group consisting of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3 and SEQ ID NO:4.

31. The isolated antibody of claim 30 wherein said antibody is selected from the group consisting of a monoclonal antibody produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7406 and a monoclonal antibody produced by a hybridoma designated by American Type Culture Collection deposit number PTA-7808.

32. The isolated antibody of claim 30 wherein said antibody comprises at least one amino acid sequence selected from the group consisting of SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7 and SEQ ID NO:8.

33. A method for treating or preventing an amyloidosis in a patient in need of said treatment or prevention comprising administering said antibody of claim 1 or claim 2 to said patient in an amount sufficient to effect said treatment or prevention.

34. The method of claim 33 wherein said amyloidosis is Alzheimer's disease or the amyloidosis of Down's sydrome.

35. An isolated antibody which binds to at least one epitope of amyloid beta protein in the brain of a patient having amyloidosis.

36. The isolated antibody of claim 35, wherein said antibody is produced by a hybridoma having an ATCC deposit number selected from the group consisting of PTA-7406 and PTA-7808.

37. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient

b. contacting said biological sample with said isolated antibody of claim 1 or claim 2 for a time and under conditions sufficient for formation of antigen/antibody complexes; and

c. detecting presence of said antigen/antibody complexes in said sample, presence of said complexes indicating a diagnosis of Alzheimer's Disease in said patient.

38. The method of claim 37 wherein said antigen is a globulomer.

39. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with an antigen for a time and under conditions sufficient for the formation of antibody/antigen complexes;

c. adding a conjugate to the resulting antibody/antigen complexes for a time and under conditions sufficient to allow said conjugate to bind to the bound antibody, wherein said conjugate comprises said isolated antibody of claim 1 or claim 2, attached to a signal generating compound capable of generating a detectable signal; and

d. detecting the presence of an antibody which may be present in said biological sample, by detecting a signal generated by said signal generating compound, said signal indicating a diagnosis of Alzheimer's Disease in said patient.

40. The method of claim 28 wherein said antigen is a globulomer.

41. A method of diagnosing Alzheimer's Disease in a

patient suspected of having Alzheimer's Disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with anti-antibody, wherein said anti-antibody is specific for said antibody of claim 1 or claim 2, for a time and under conditions sufficient to allow for formation of anti-antibody/antibody complexes, said complexes containing antibody present in said biological sample;

c. adding a conjugate to resulting anti-antibody/antibody complexes for a time and under conditions sufficient to allow said conjugate to bind to bound antibody, wherein said conjugate comprises an antigen, which binds to a signal generating compound capable of generating a detectable signal; and

d. detecting a signal generated by said signa generating compound, said signal indicating a diagnosis of Alzheimer's Disease in said patient.

42. A vaccine comprising: a) said isolated antibody of claim 1, said isolated antibody of claim 2 or a combination thereof and b) a pharmaceutically acceptable adjuvant.

43. A method of identifying compounds suitable for active immunization of a patient predicted to develop Alzheimer's Disease comprising the steps of:

a) exposing one or more compounds of interest to said isolated antibody of claim 1 or claim 2, for a time and under conditions sufficient for said one or more compounds to bind to said isolated antibody of claim 1 or claim 2; and

b) identifying those compounds which bind to said isolated antibody of claim 1 or claim 2, said identified compounds to be used in active

immunization in a patient predicated to develop Alzheimer's Disease.

44. A kit comprising: a) said isolated antibody of claim 1 or claim 2 and b) a conjugate comprising an antibody attached to a signal-generating compound, wherein said antibody of said conjugate is different from said isolated antibody.

45. A kit comprising: a) an anti-antibody to said isolated antibody of claim 1 or claim 2 and b) a conjugate comprising an antigen attached to a signal-generating compound.

46. The kit of claim 46 wherein said antigen is a globulomer.

Figure 1a

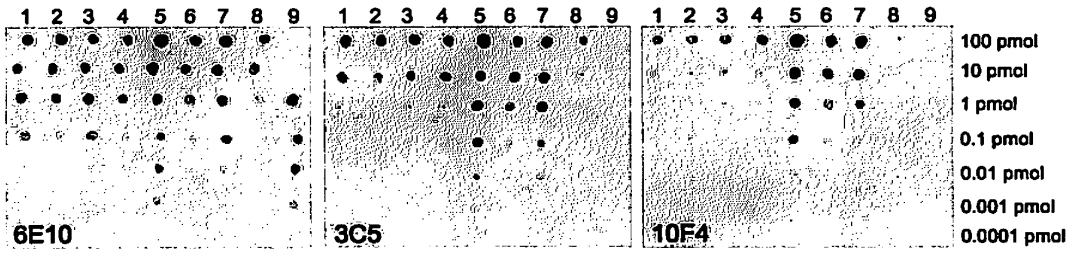


Figure 1b

	Aβ(1-42) monomer in 0.1% NH_4OH	Aβ(1-40) monomer in 0.1% NH_4OH	Aβ(1-42) monomer in 0.1% NaOH	Aβ(1-40) monomer in 0.1% NaOH	Aβ(1-42) globulomer	Aβ(12-42) globulomer	Aβ(20-42) globulomer	Aβ(1-42) fibril	sAPPα
6E10	41	132	41	196	1	225	16	1216	0.8
10F4	645	1429	455	769	1	11	9	12500	> 100
3C5	341	577	288	306	1	47	6	3488	> 1000

Figure 2a

patient number	diagnosis	gender	age at death	post-mortem time	Braak & Braak index	CERAD index	brain sample region
RZ55	AD	male	80	12	V	C	frontal cortex
RZ119	AD	male	76	24	VI	C	frontal cortex
RZ122	AD	female	83	14	V-VI	C	frontal cortex
RZ296	AD	male	88	4	V	C	frontal cortex
RZ307	AD	female	78	21	VI	C	frontal cortex
ABS 0504009	AD	male	88	3	n.d.	n.d.	frontal cortex
RZ145	non-demented	female	86	20	I	0	frontal cortex
RZ342	non-demented	female	84	22	II	0	frontal cortex

Figure 2b

SELDI-MS analysis	Alzheimer's disease brain												age matched control brain			
	RZ55		RZ119		RZ122		RZ296		RZ307		ABS 0504009		RZ145		RZ342	
	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])	Aβ(1-40) [a.u.]	Aβ(1-42 [a.u.])
6E10	6074	685	370	826	284	1101	161	1030	8736	1688	66	933	38	272	76	491
3C5	1667	110	13	21	58	652	32	507	2048	233	17	110	2	4	15	54
10F4	2655	303	17	47	69	684	39	818	4012	856	48	395	2	34	47	286
IgG2b	10	1	2	3	2	10	2	4	9	4	3	9	1	4	1	1

Figure 2c

SELDI-MS analysis	Alzheimer's disease brain												age matched control brain			
	RZ55		RZ119		RZ122		RZ296		RZ307		ABS 0504009		RZ145		RZ342	
	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)	Aβ(1-40) % Aβ of 6E10 IP	Aβ(1-42) % Aβ of 6E10 IP)
3C5	27	16	4	3	20	59	20	49	30	14	26	12	5	1	20	11
10F4	44	44	5	8	24	62	24	60	60	51	70	42	5	13	62	58
IgG2b	0	0	1	0	1	1	1	0	0	0	5	1	3	1	1	0

Figure 2d

Western Blot analysis	Alzheimer's disease brain						age matched control brain	
	RZ55 Aβ [a.u.]	RZ119 Aβ [a.u.]	RZ122 Aβ [a.u.]	RZ296 Aβ [a.u.]	RZ307 Aβ [a.u.]	ABS 0504009 Aβ [a.u.	RZ145 Aβ [a.u.]	RZ342 Aβ [a.u.]
6E10	229402	146045	151948	172822	242537	123837	42788	61855
3C5	44195	5624	35957	49785	47228	10662	169	3993
10F4	97036	44398	88681	103612	120196	44366	963	29300
IgG2b	754	109	601	1594	233	111	45	91

Figure 2e

Western Blot analysis	Alzheimer's disease brain						age matched control brain	
	RZ55 % Aβ of 6E10 IP	RZ119 % Aβ of 6E10 IP	RZ122 % Aβ of 6E10 IP	RZ296 % Aβ of 6E10 IP	RZ307 % Aβ of 6E10 IP	ABS 0504009 % Aβ of 6E10 IP	RZ145 % Aβ of 6E10 IP	RZ342 % Aβ of 6E10 IP
3C5	19	4	24	29	19	9	0	6
10F4	42	30	58	60	50	36	2	47
IgG2b	0	0	0	1	0	0	0	0

Figure 3a

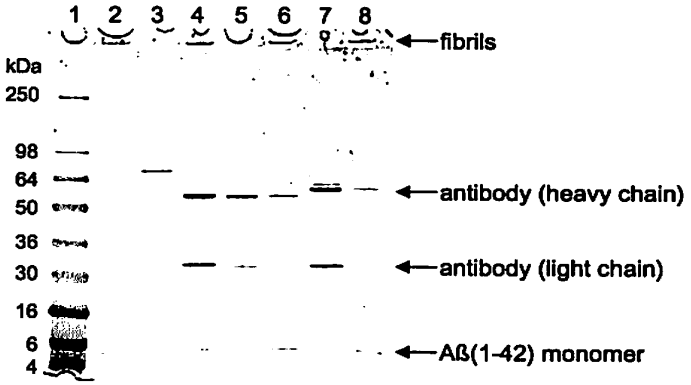


Figure 3b

		trace OD x mm [a.u.]	antibody bound to fibrils [%]
6E10	supernatant	0.007	97
	pellet	0.233	
3C5	supernatant	0.111	34
	pellet	0.057	
10F4	supernatant	0.160	23
	pellet	0.047	

Figure 4a

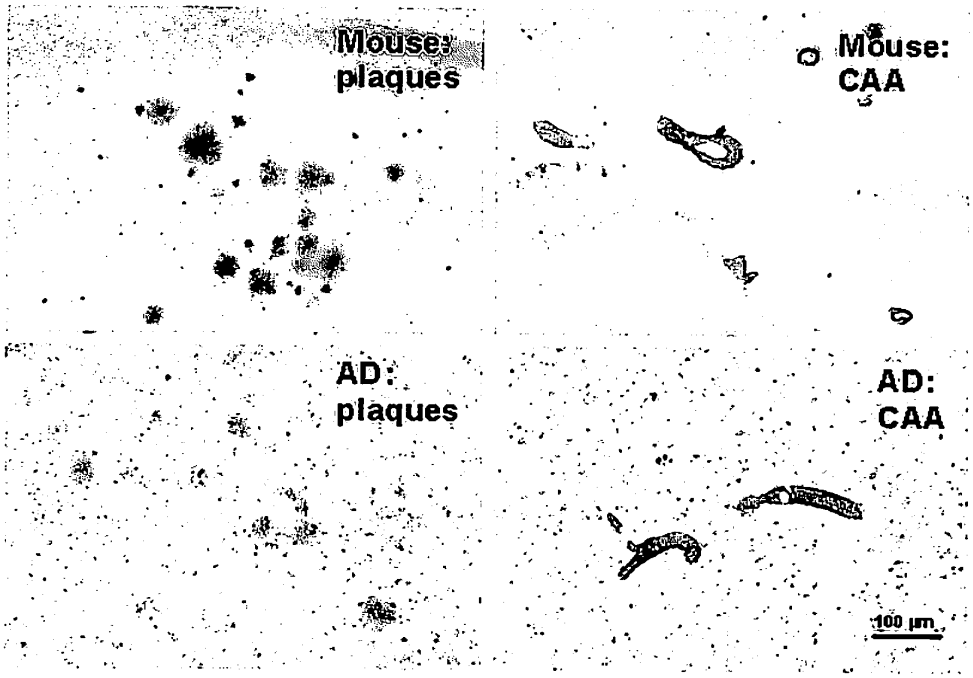


Figure 4b

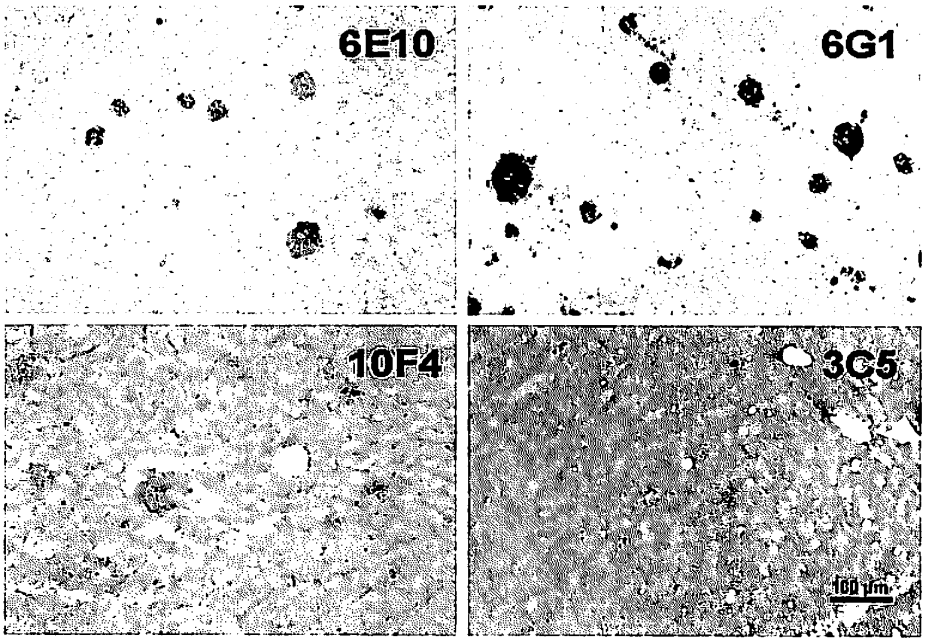


Figure 4c

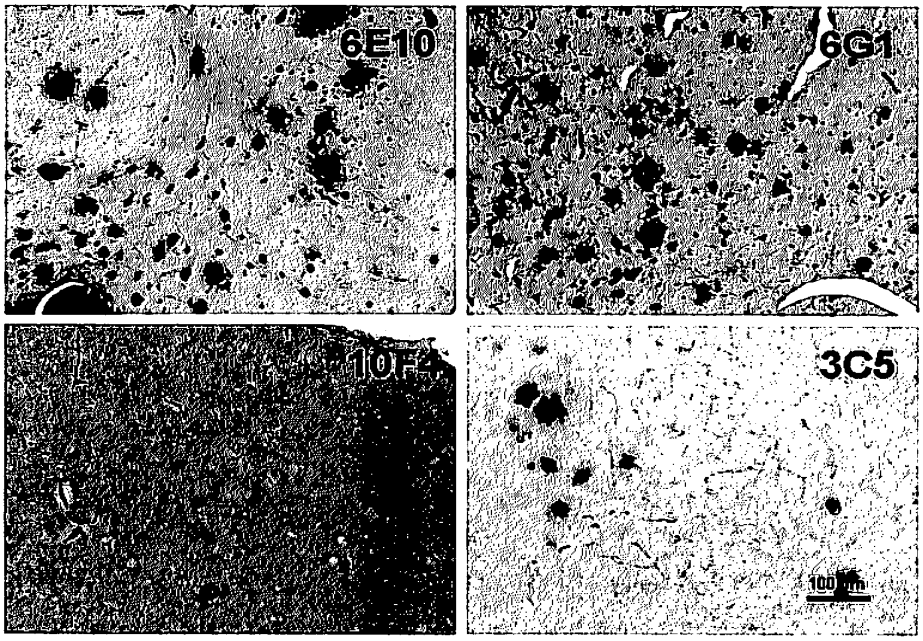


Figure 4d-g

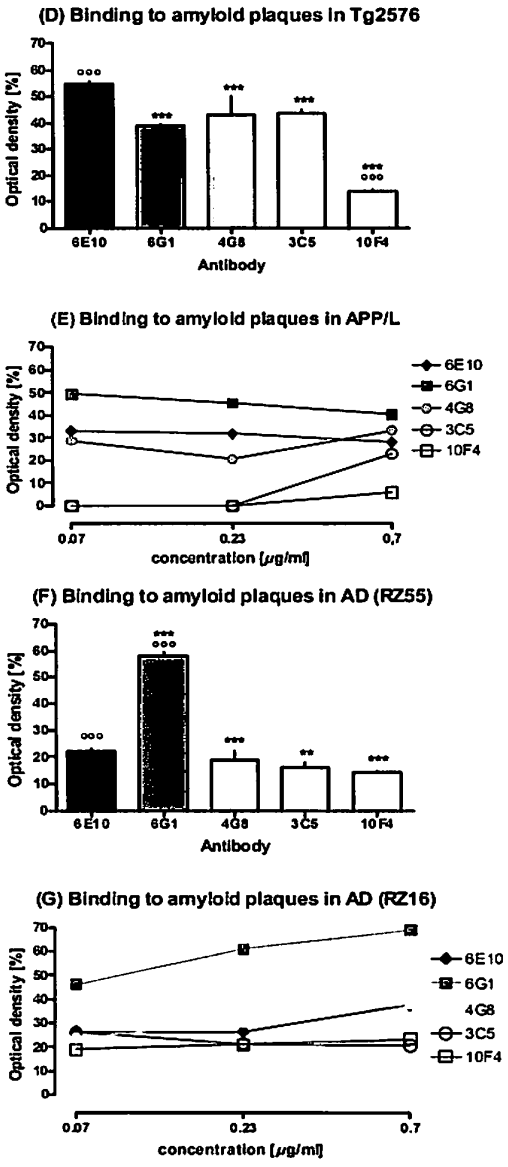

(D) Binding to amyloid plaques in Tg2576
Optical density [%]
Antibody
6E10
6G1
4G8
3C5
10F4
(E) Binding to amyloid plaques in APP/L
concentration [µg/ml]
0.07
0.23
0.7
(F) Binding to amyloid plaques in AD (RZ55)
(G) Binding to amyloid plaques in AD (RZ16)

Figure 4h

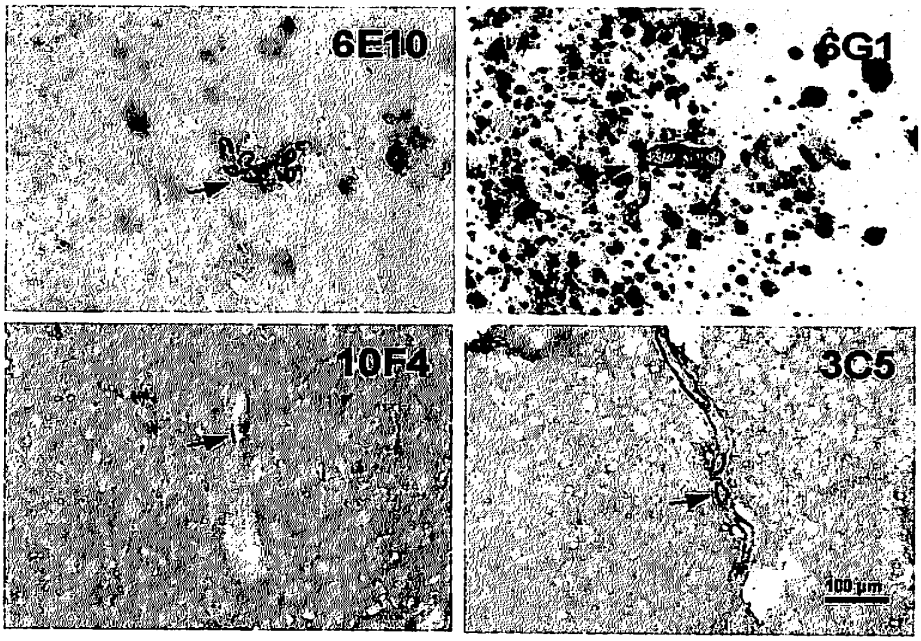


Figure 5a

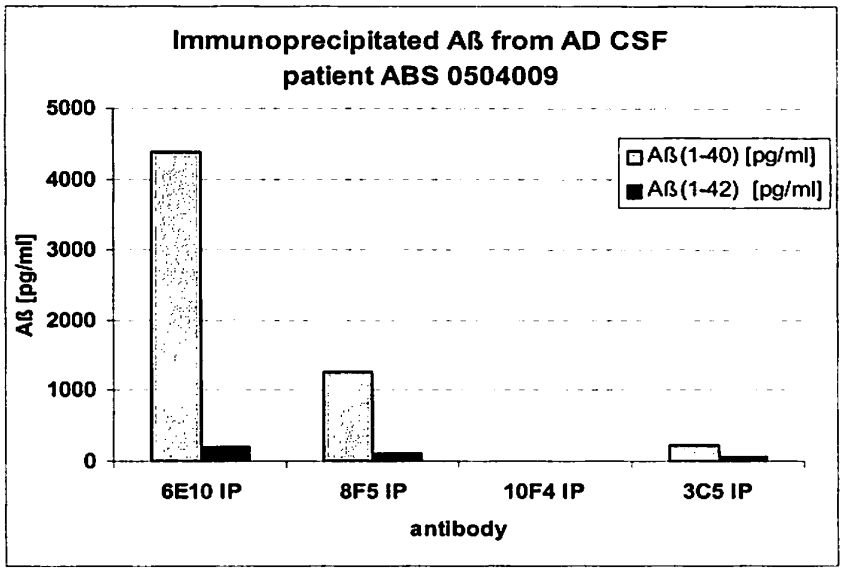


Figure 5b

patient #	antibody	% Aβ(1-40) of 6E10 IP	% Aβ(1-42) of 6E10 IP
ABS 0504009	8F5	29	55
	10F4	0	0
	3C5	5	38

Figure 5c

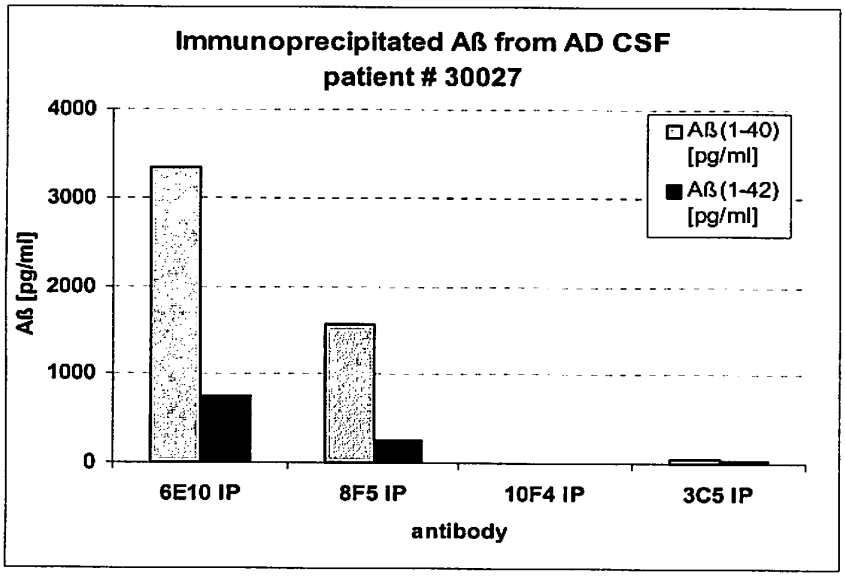


Figure 5d

patient #	antibody	% Aβ(1-40) of 6E10 IP	% Aβ(1-42) of 6E10 IP
30027	8F5	47	34
	10F4	0	0
	3C5	2	5

Figure 5e

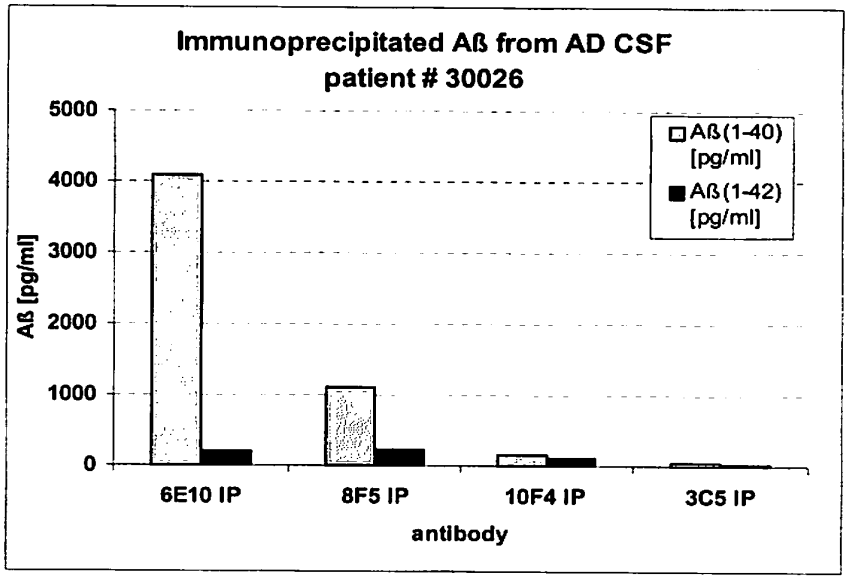


Figure 5f

patient #	antibody	% Aβ(1-40) of 6E10 IP	% Aβ(1-42) of 6E10 IP
30026	8F5	27	112
	10F4	4	56
	3C5	1	9

Figure 5g

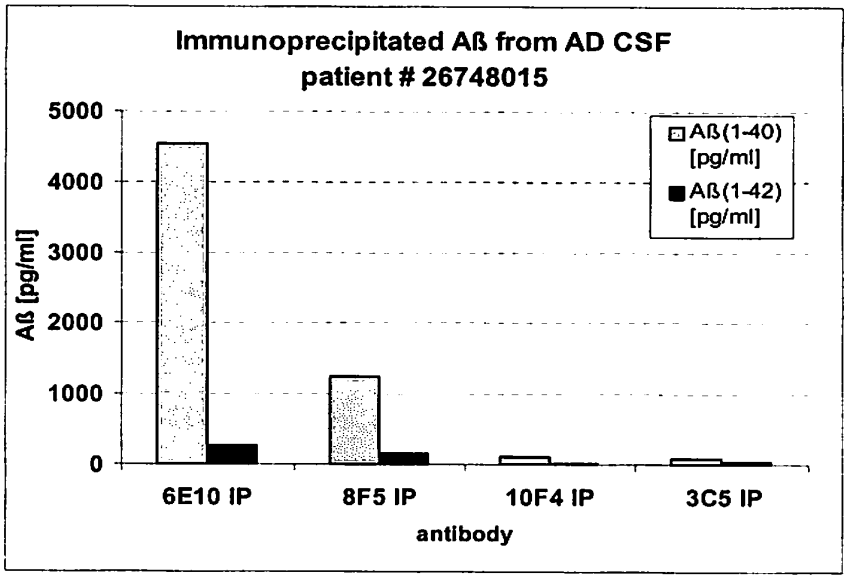


Figure 5h

patient #	antibody	% Aβ(1-40) of 6E10 IP	% Aβ(1-42) of 6E10 IP
26748015	8F5	28	58
	10F4	3	13
	3C5	2	17

Figure 5i

			post-mortem CSF sample		pre-mortem CSF sample	
patient #	diagnosis	gender	patient age at death	post-mortem time (h)	patient age at sample taken	Mini Mental State Examination (MMSE) score
ABS 0504009	AD	male	88	3	n.a.	n.d.
30027	AD	male	n.a.	n.a.	72	14
30026	AD	male	n.a.	n.a.	70	22
26748015	AD	female	n.a.	n.a.	48	11

Figure 6a

SEQ ID NO:1 (VH ML45-3C5)

GATGTGCAGCTTCAGGAGTCGGGACCTGGCCTGGTGAAACCTTCTCAGTCTCTGT
CCCTCACCTGCACTGTCGCTGGCTCCTCAATCACCAGTCATTATGCCTGGAACTGG
ATCCGGCAGTTTCCAGGAAACAAACTGGAGTGGATGGGCTACATAGACTATAGT
GGTAGCACTCGCTACCTCCCCTCTCTCAAAAGTCGAATCTCTATCACTCGAGACA
CATCCAAGAACCAGTTCTTCCTGCAGTTGAATTCTGTGACTACTGAGGACACAGC
CACATATTACTGTGCAAGGGGTAGTGGTTATTTCTATGGTATGGACTACTGGGGT
CAAGGAACCTCAGTCACCGTCTCCTCA

Figure 6b

SEQ ID NO:2 (VL ML45-3C5)

GACATCCAGATGAACCAGTCTCCATCCAGTCTGTCTCCATCCCTTGGAGACACAA
TTACCATCACTTGCCATGCCAGTCAGAACATTAATGTCTGGTTAAGCTGGTACCA
GCAGAAACCAGGAAATATTCCTAAACTATTGATCTATAAGGCTTCCAACTTGCAC
ACAGGCGTCCCATCAAGGTTTAGTGGCAGTGGATCTGGAATAGGTTTTACATTAA
CCATCCGCAGCCTGCAGCCTGAAGACATTGCCACTTACTTCTGTCAACAGGGTCA
AAGTTATCCGTACACGTTCGGAGGGGGGACTAAGCTGGAAATAAAACGG

Figure 6c

SEQ ID NO:3 (VH ML43-10F4)

CAGGTCCAGCTGCAGCAGTCTGGAGCTGAGCTGGTAAGGCCTGGGACTTCAGTGA
GGGTGTCCTGCAAGGCTTCTGGATACGCCTTCACTAATTACTTGATAGAGTGGGT
AAAGCAGAGGCCTGGACAGGGCCTTGAGTGGATTGGAGTGATTAATCCTGGAAG
TGGTGATACTAACTACAATGAGAATTTCAAGGGCAAGGCAACACTGACTGCAGA
CAAATCCTCCAGCACTGCCTACATGCACCTCAGCAGCCTGACATCTGATGACTCT
GCGGTCTATTTCTGTACAAGAGGCGTGATTACGACGGGTTTTGACTACTGGGGCC
AAGGCACCACTCTCACAATCTCCTCA

Figure 6d

SEQ ID NO:4 (VL ML43-10F4)

GACATCCAGATGACTCAGTCTCCAGCCTCCCTATCTGCATCTGTGGGAGAAACTG
TCACCATCACATGTCGAGCAAGTGGGAATATTCACAATTATTTAGCATGGTATCA
GCAGAAACAGGGAAAATCTCCTCAGCTCCTGGTCTATAATGCAAAAACCTTAGCA
GATGGTGTGCCATCAAGGTTCAGTGGCAGTGGATCAGGAACACAATATTCTCTCA
AGATCAACAGCCTGCAGCCTGAAGATTTTGGGAGTTATTACTGTCAACATTTTTG
GAGTAGTCCTCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAACGG

Figure 7a

SEQ ID NO:5 (VH ML45-3C5)

DVQLQESGPGLVKPSQSLSLTCTVAGSSITSHYAWNWIRQFPGNKLEWMGYIDYSGS
TRYLPSLKSRISITRDTSKNQFFLQLNSVTTEDTATYYCARGSGYFYGMDYWGQGTS
VTVSS

Figure 7b

SEQ ID NO:6 (VL ML45-3C5)

DIQMNQSPSSLSPSLGDTITITCHASQNINVWLSWYQQKPGNIPKLLIYKASNLHTGVP
SRFSGSGSGIGFTLTIRSLQPEDIATYFCQQGQSYPYTFGGGTKLEIKR

Figure 7c

SEQ ID NO:7 (VH ML43-10F4)

QVQLQQSGAELVRPGTSVRVSCKASGYAFTNYLIEWVKQRPGQGLEWIGVINPGSGD
TNYNENFKGKATLTADKSSSTAYMHLSSLTSDDSAVYFCTRGVITTGFDYWGQGTTL
TISS

Figure 7d

SEQ ID NO:8 (VL ML43-10F4)

DIQMTQSPASLSASVGETVTITCRASGNIHNYLAWYQQKQGKSPQLLVYNAKTLADG
VPSRFSGSGSGTQYSLKINSLQPEDFGSYYCQHFWSSPRTFGGGTKLEIKR

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
10 July 2008 (10.07.2008)

PCT

(10) International Publication Number
WO 2008/081008 A1

(51) International Patent Classification:
C07K 16/18 (2006.01) *A61P 25/28* (2006.01)
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/EP2008/000053

(22) International Filing Date: 7 January 2008 (07.01.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

60/878,831	5 January 2007 (05.01.2007)	US
07000211.8	5 January 2007 (05.01.2007)	EP
60/934,291	11 June 2007 (11.06.2007)	US
07020341.9	17 October 2007 (17.10.2007)	EP

(71) Applicant *(for all designated States except US)*: **UNIVERSITY OF ZURICH** [CH/CH]; Prorektorat Forschung, Rämistrasse 71, CH-8006 Zürich (CH).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **NITSCH, Roger** [DE/CH]; Langwisstr. 27, CH-8126 Zumikon (CH). **HOCK, Christoph** [DE/CH]; Rietstrasse 43, CH-8703 Erlenbach (CH). **ESSLINGER, Christoph** [DE/CH]; Zollikerstrasse 3, CH-8008 Zurich (CH). **KNOBLOCH, Marlen** [CH/CH]; Kyburgstrasse 26, CH-8037 Zurich (CH). **TISSOT, Kathrin** [DE/CH]; Segantinistrasse 35, CH-8049 Zürich (CH). **GRIMM, Jan** [DE/CH]; Bürglistr. 16, CH-8600 Düebendorf (CH).

(74) Agent: **STEINECKE, Peter**; Müller Fottner Steinecke, P.O. Box 31 10 40, 80102 Munich (DE).

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

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

Published:
— *with international search report*
— *with sequence listing part of description published separately in electronic form and available upon request from the International Bureau*

(54) Title: METHOD OF PROVIDING DISEASE-SPECIFIC BINDING MOLECULES AND TARGETS

(57) Abstract: Provided are novel specific binding molecules, particularly human antibodies as well as fragments, derivatives and variants thereof that recognize neoepitopes of disease-associated proteins which derive from native endogenous proteins but are prevalent in the body of a patient in a variant form and/or out of their normal physiological context. In addition, pharmaceutical compositions comprising such binding molecules, antibodies and mimics thereof and methods of screening for novel binding molecules, which may or may not be antibodies as well as targets in the treatment of neurological disorders such as Alzheimer's disease are described.

WO 2008/081008 A1

METHOD OF PROVIDING DISEASE-SPECIFIC BINDING MOLECULES AND TARGETS

Field of the Invention

[0001] The present invention relates to novel specific binding molecules, particularly human antibodies as well as fragments, derivatives and variants thereof that recognize disease-associated epitopes, including neoepitopes, of proteins which derive from native endogenous proteins, and which are prevalent in the body of a patient in a variant form and/or out of their normal physiological context. In addition, the present invention relates to pharmaceutical compositions comprising such binding molecules, antibodies and mimics thereof, and to methods of screening for novel binding molecules, which may or may not be antibodies, targets and drugs in the treatment of various disorders, in particular neurological disorders such as Alzheimer's disease, amyloidoses and beta-amyloid pathology.

BACKGROUND OF THE INVENTION

[0002] The success in generating monoclonal antibodies rests on the efficient and selective fusion of antigen-stimulated B cells with a murine myeloma cell line followed by selection of stable antibody producing hybrids as originally described by Köhler and Milstein, Nature 256 (1975), 495-497. However, the therapeutic utility of murine based antibodies in human is hampered by the human anti-mouse antibody (HAMA) response in view of their non-human origin. Approaches for making human or human-like monoclonal antibodies became available through genetic engineering. However, the methods hitherto available suffer from the drawback that they are not suitable to produce antibodies with the characteristics of those produced in the course of a physiological human immune response. Furthermore, such antibodies may not be specific enough because of cross-reactivity with other proteins and/or the target protein in context with normal physiological function. In case of Alzheimer's or Parkinson's disease, for example, antibodies that also cross-react with high affinity with physiological derivatives of amyloid precursor protein (APP) or

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6437

alpha synuclein are considered to exhibit side effects related to the normal functions of the physiologic target structures. In this respect, an undesired autoimmune disease would downrightly be induced – a hardly calculable risk in the conceptual design of active immunization experiments employing protein structures that, in variant form, also occur physiologically. Side effects not related to the target structure are, for example, anaphylactic reactions, as are to be expected as undesired and dreaded side effects of the systemic administration of exogenous proteins. According to recent findings, this can also be the case in so-called humanized antibodies, which originally stem from non-human organisms, usually from mice. On the other hand, active immunization with pathological relevant antigens bears the considerable risk of patients developing antibodies and T cell responses which also recognize physiological variants of such proteins and in consequence lead to a dangerous and uncontrollable autoimmune response.

[0003] Thus, there is a need of providing agents which are specific for a target involved in a disorder and which are tolerated by the human body.

SUMMARY OF THE INVENTION

[0004] An object of the present invention is a method for identifying, validating and producing diagnostically and therapeutically useful binding molecules, in particular antibodies that are directed against pathologic variants of endogenous proteins. More specifically, the present invention relates to a method of isolating a disease-associated protein-specific binding molecule comprising:

[0005] (a) subjecting a sample obtained from a patient who is symptom-free, or who is clinically unusually stable, but who is affected with or at risk of developing a disorder to a specimen of pathologically altered cells or tissues with predetermined pathological characteristics; and

[0006] (b) identifying and optionally isolating a binding molecule which binds to said specimen but not to corresponding cells or tissues without such pathological characteristics as it may be derived from a healthy subject.

[0007] Known is the fact that, in case of autoimmune diseases, antibodies are directed against autologous cells and proteins or other compounds such as glycolipids expressed by said cells while evading the known tolerance mechanisms. Also known is the fact that, in case of endogenous neoplastic developments, a cellular and humoral immunity to the

neoplastic cells can develop and can thus effect an endogenous immunological protection mechanism against neoplastic tissue degeneration.

[0008] The present invention makes use of the surprising finding that antibodies can also be directed against pathophysiologically relevant variants of endogenous proteins, in particular against neoepitopes, which are formed due to pathologically altered transcription, translation, or post-transcriptional or post-translational modification, or proteolytic processing, or aggregation. Such antibodies are directed against endogenous proteins which, owing to their new structure that deviates from the normal physiology, become pathophysiologically relevant by means of developing pathological effects. For reasons of immune tolerance, the antibodies connected with the corresponding immune response to neoepitopes in such pathological variants do not normally exhibit any cross reactions against the physiologically functional proteins, however, as opposed to the case of autoimmune diseases. This is because the formation of potentially cross-reactive antibodies is specifically suppressed by the known tolerance mechanisms, whereas the development of an immune response to pathological neoepitopes can escape tolerance.

[0009] Hence, the present invention relates to a novel approach of identifying diagnostically, therapeutically, and preventively active binding molecules, especially antibodies and antibody fragments from clinically preselected human subjects by means of interaction with identifiable pathological structures.

[0010] The present invention is thus directed to antibodies or antigen-binding fragments and similar antigen binding molecules which are capable of recognizing epitopes, including neoepitopes, of disease-associated proteins which derive from native endogenous proteins and are prevalent in the body of a patient in a variant form, *e.g.* as a pathological protein and/or out of their normal physiological context. Furthermore, the present invention relates to compositions comprising said antibodies and to immunotherapeutic and immunodiagnostic methods using the same.

[0011] Furthermore, in antibody identification, the method according to the present invention can do without previous hypothesis on the identity of its molecular target structure, solely by means of its association with pathologically relevant structures. Besides the possibility of thus identifying molecular target structures hitherto unknown for specific diseases, a further advantage of antibodies that are exclusively directed against pathological structures is based on the fact that their pharmacodynamic availability is not negatively influenced by binding to non-diseased tissues in such a way that the antibody is

buffered with respect to its concentration and sink effects thus hampering the determination of therapeutically effective concentrations. Furthermore, the antibody and binding molecules of the present invention are preferably characterized in that they react with the variant form of the disease-associated protein *in vivo* or with a cell or cell membrane, and on a section of the pathologically characterized diseased tissue, respectively, but not or to a significantly lesser extent with the physiological variant of the cognate protein; see also, *e.g.*, Example 2.

[0012] Since the present invention enables identifying and isolating molecular target structures in diseased cells and tissues, a further embodiment concerns the antigen and pathological protein, *i.e.* disease-associated protein, respectively, which is bound by the neoepitope-specific antibody of the present invention.

[0013] A particularly preferred embodiment is a human antibody or antigen-binding fragment thereof which demonstrates the immunological binding characteristics of any of the antibody characterized by the variable regions V_H and/or V_L as set forth in Tables 2 and 3, *infra*. Alternatively, the antibody is a humanized, xenogeneic, or a chimeric human-murine antibody, the latter being particularly useful for diagnostic methods and studies in animals. Therapeutic compositions including the antibody or active fragments thereof, or agonists and cognate molecules, or alternately, antagonists of the same, and methods of use of such compositions in the prevention, diagnosis or treatment of a disease using these compositions are also included, wherein an effective amount of the composition is administered to a patient in need of such treatment.

[0014] The antigen-binding fragment of the antibody can be a single chain Fv fragment, an F(ab') fragment, an F(ab) fragment, and an $F(ab')_2$ fragment, or any other antigen-binding fragment. In a specific embodiment, *infra*, the antibody or fragment thereof is a human IgG isotype antibody.

[0015] Naturally, the present invention extends to the immortalized human B memory lymphocyte and B cell, respectively, that produces the antibody having the distinct and unique characteristics as defined below.

[0016] The present invention also relates to polynucleotides encoding at least a variable region of an immunoglobulin chain of the antibody of the invention. Preferably, said variable region comprises at least one complementarity determining region (CDR) of the V_H and/or V_L of the variable region as set forth in Tables 2 and 3, *infra*. A corresponding set of CDRs is given in Table 4, *infra*.

[0017] Accordingly, the present invention also encompasses vectors comprising said polynucleotides and host cells transformed therewith as well as their use for the production of an antibody and equivalent binding molecules which are specific for neoepitopes that are indicative and/or causative for a disorder, in particular for a disorder of the brain such as Alzheimer's disease and Parkinson disease.

[0018] The antibody, immunoglobulin chain(s), binding fragments thereof and antigen binding to said antibody can be used in pharmaceutical and diagnostic compositions for immunotherapy and diagnosis, respectively. The use of the foregoing compositions in the preparation of a medicament is however preferred.

[0019] Hence, it is a particular object of the present invention to provide methods for treating or preventing a neurological disorder characterized by abnormal accumulation and/or deposition of a protein in the central nervous system without interfering with the natural function of the respective protein. The methods comprise administering an effective concentration of an antibody or antibody derivative to the subject where the antibody binds to the pathological form of the protein or the protein deposit with a substantially higher affinity than to the normal physiological form of the protein. In a preferred embodiment, the present invention provides methods for treating or preventing or slowing the onset of diseases associated with the accumulation and deposition of the amyloid beta peptide in a subject, such as Alzheimer's disease, Down's syndrome, mild cognitive impairment, cerebral amyloid angiopathy, vascular dementia, multi-infarct dementia. The methods comprise administering an effective concentration of an antibody or antibody derivative to the subject where the antibody binds to the pathological form of the protein or the protein deposit with higher affinity than to the normal physiological form of the protein. Similar therapeutic approaches are envisaged for the treatment of Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, cystic fibrosis, Gaucher's disease and the like.

[0020] Further embodiments of the present invention will be apparent from the description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] **Fig. 1:** Antibody against beta-amyloid. **A**: Human antibodies. **B**: Control staining with known antibody against human beta-amyloid. Clinically unusually stable patients

with Alzheimer's disease contain antibodies to beta-amyloid plaques. Immunohistochemical staining with antibodies from clinically unusually stable patients on brain sections obtained from patients with pathologically confirmed Alzheimer's disease reveals antibodies that bind to beta-amyloid plaques confirmed by a known antibody against human beta-amyloid.

[0022] **Fig. 2:** Antibody against neurofibrillary tangles. **A**: Human antibodies. **B**: Control staining with known antibody against human tau. Healthy human subjects contain antibodies to neurofibrillary tangles. Immunohistochemical staining with antibodies from healthy subjects on brain sections obtained from patients with pathologically confirmed Alzheimer's disease reveals antibodies that bind to neurofibrillary tangles confirmed by a known antibody against human tau.

[0023] **Fig. 3:** Antibody against dystrophic neurites. **A**: Human antibodies. **B**: Control staining with known antibody against human tau. Healthy human subjects contain antibodies to dystrophic neurites. Immunohistochemical staining with antibodies from healthy subjects on brain sections obtained from patients with pathologically confirmed Alzheimer's disease reveals antibodies that bind to dystrophic neurites.

[0024] **Fig. 4:** Antibody against beta-amyloid. The figure shows specific binding of recombinant human NI-101.11 antibody that was isolated from a clinically unusually stable Alzheimer's disease patient to brain beta-amyloid plaques. Brain sections obtained from a patient with neuropathologically confirmed Alzheimer's disease were stained with recombinant human antibody at the indicated concentrations. Antibody binding to beta-amyloid plaques with concentrations of 50 pM suggest high affinity binding.

[0025] **Fig. 5:** Binding of recombinant human NI-101.11 antibody to beta-amyloid plaques is not competed by linear synthetic N-terminal Abeta polypeptides. Binding of the recombinant antibody against brain beta-amyloid (0.5 nM) cannot be competed by N-terminal Abeta-derived polypeptide representing positions 1 to 16 at concentrations up to 1 μM.

[0026] **Fig. 6:** Recombinant human NI-101.11 antibody recognizes a conformational Abeta epitope that is not present in monomeric Abeta. Binding of NI-101.11 to beta-amyloid plaques on brain sections can be competed by Abeta1-42 fibrils but not linear synthetic Abeta1-42 monomers.

[0027] **Fig. 7:** Recombinant human NI-101.11 antibody does not bind to linear, monomeric synthetic Abeta on Western blots. Preparations of monomeric Abeta were

separated by non-denaturing PAGE. Blotted protein was probed with human recombinant antibody against beta-amyloid and control antibodies against N- terminal linear Abeta sequences (6E10). No binding of NI-101.11 to monomeric Abeta was detected. This observation suggests that the antibody recognizes a conformational Abeta epitope.

[0028] **Fig. 8:** Human NI-101.11 antibody binds artificial amyloid fibrils prepared from synthetic Abeta1-42 peptides. Synthetic Abeta fibrils or monomeric synthetic Abeta coated onto ELISA plates at equal coating densities were incubated with recombinant human antibodies against brain beta-amyloid at the indicated concentrations. Binding activity of human antibody against brain beta- amyloid to artificial amyloid fibrils (open squares) is more than 100 times higher as compared to monomeric Abeta (filled squares). Control antibody 22C4 preferentially binds to monomeric Abeta (filled cicles), and less well to fibrils (open circles). This suggests that NI-101.11 recognizes a conformational epitope which is also present on artificial amyloid fibrils prepared from synthetic Abeta peptides.

[0029] **Fig. 9:** Absent cross-reactivity of recombinant human NI-101.11 antibody to cellular full-length APP or with any of its physiological derivatives occurring in cultured cells. In contrast to the control antibody (6E10) that binds to cell-surface APP, binding of NI-101.11 to full-length APP present at cellular surfaces is absent. These data demonstrate absent cross-reactivity of NI-101.11 to physiological, cellular full-length APP.

[0030] **Fig. 10A-C:** Absence of binding of NI-101.11 to monomeric Abeta via size exclusion chromatography. Figures 10A and 10B show no binding of NI-101.11 or an unrelated control antibody to monomeric FITC-labeled Abeta1-42 while Figure 10C shows prominent binding of antibody 22C4 that recognizes a linear epitope present in the C-terminus of Abeta.

[0031] **Fig. 11:** Competition ELISA showing that binding of antibody 6E10, an antibody directed against a linear epitope at the N-terminus of Abeta could be completely blocked upon pre-incubation with excess concentrations of monomeric Abeta peptides while pre-incubation with excess concentrations of these monomeric Abeta peptide preparations did not abolish NI-101.11 binding.

[0032] **Fig. 12:** Binding of NI-101.13A and NI-101.13B to brain sections obtained from Tg2676 transgenic mouse model of Alzheimer's disease.

[0033] **Fig. 13:** ELISA showing preferential binding of NI-101.13A and NI-101.13B to artificial amyloid fibrils as compared to monomeric Abeta.

[0034] **Fig. 14A-B:** Figure 14A shows the binding of recombinant NI-101.12 to synthetic Abeta1-42 peptide via ELISA. Figure 14B shows NI-101.12 binding was competed by excess Abeta1-42 peptide.

[0035] **Fig. 15:** Recombinant human NI-101.11 antibody against brain beta-amyloid crosses the blood brain barrier in a transgenic mouse model of Alzheimer's disease, and binds to brain beta-amyloid plaques *in vivo*.

[0036] **Fig. 16A-B:** Recombinant human NI-101.11 antibody improves abnormal cognitive behavior in a transgenic mouse model of Alzheimer's disease. 24 months old arcAbeta mice were treated weekly i.p. with 3 mg/kg antibody for 2 months. Y-maze behavioral testing was performed before and after completion of the treatment.

[0037] **Fig. 17:** Blood-brain barrier penetration and decoration of amyloid plaques by peripherally administered NI-101.11. NI-101.11 can cross the blood-brain barrier and bind to beta-amyloid deposits in NI-101.11 treated mice (left panel) whereas no such staining is visible in animals treated with the human control antibody (right panel).Recombinant human NI-101.11 antibody reduces brain beta-amyloid plaque load after systemic treatment for two months.

[0038] **Fig. 18:** Passive immunization with NI-101.11 reduces beta-amyloid load in arcAbeta mice. (A, B) Thioflavin S and Congo Red plaque load analyses reveal significant reductions of more than 50% compared to the control antibody treated animals (Mann-Whitney U; p=0.02 for cortex, p=0.009 for hippocampus for ThioS and p=0.009 for cortex and p=0.04 for hippocampus for Congo Red analysis). Scale bar: 200 μm.(C-E) Thioflavin S analysis reveals a significant reduction in beta-amyloid burden (C), number of beta-amyloid plaques (D) and average plaque size (E) in NI-101.11 treated arcAbeta mice compared to control treated animals. Mann-Whitney U statistics: p=0.02 for plaque area cortex; p=0.009 for plaque area hippocampus; p=0.047 for plaque number cortex; p=0.047 for plaque number hippocampus; p=0.009 for plaque size cortex; p=0.009 for plaque number hippocampus.

[0039] **Fig. 19:** Reduced beta-amyloid load is accompanied by decreased astrocytosis and microgliosis A) Quantification of anti-GFAP staining revealed a significant reduction in the number of reactive astrocytes in the cortex of NI-101.11 treated arcAbeta mice when compared to control treated transgenics. B) Quantification of Iba-1 staining showed a trend towards a reduced number of activated microglia in NI-101.11 treated mice in cortex and hippocampus. Scale bar: 200μm.

[0040] **Fig. 20:** No increase of brain microhemorrhages after two months of treatment with recombinant human NI-101.11 antibody. 24 months old arcAbeta mice with proven massive congophilic amyloid angiopathy were treated weekly i.p. with 3 mg/kg antibody for 2 months. Representative picture of a brain microhemorrhage in arcAbeta mice revealed by Perl's Prussian blue staining (left). Quantitative analysis demonstrates a significantly elevated frequency of micorhemorrhages in arcAbeta transgenic mice compared to their wildtype littermates. Chronic treatment with NI-101.11 did not result in increased frequency of micorhemorrhages. Scale bar: 20μm

[0041] **Fig. 21:** Recombinant human NI-101.11 antibody inhibits the formation of synthetic Abeta fibrils *in vitro*. The effect of recombinant human NI-101.11 antibody on the formation of Abeta fibrils was assayed by measuring Thioflavin S bound to aggregated Abeta by fluorescence analysis.

[0042] **Fig. 22:** Antibody-mediated dose-dependent phagocytosis of FITC-Abeta1-42 fibrils by BV-2 microglial cells was measured upon inhibition of the scavenger receptor system. NI-101.11 triggers potent dose-dependent Fcgamma receptor-mediated phagocytosis of Abeta fibrils.

DETAILED DESCRIPTION OF THE INVENTION

I. Definitions

[0043] It is to be noted that the term "a" or "an" entity refers to one or more of that entity; for example, "an antibody," is understood to represent one or more antibodies. As such, the terms "a" (or "an"), "one or more," and "at least one" can be used interchangeably herein.

[0044] As used herein, the term "polypeptide" is intended to encompass a singular "polypeptide" as well as plural "polypeptides," and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term "polypeptide" refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, "protein," "amino acid chain," or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of "polypeptide," and the term "polypeptide" may be used instead of, or interchangeably with any of these terms.

[0045] The term "polypeptide" is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non-naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0046] A polypeptide of the invention may be of a size of about 3 or more, 5 or more, 10 or more, 20 or more, 25 or more, 50 or more, 75 or more, 100 or more, 200 or more, 500 or more, 1,000 or more, or 2,000 or more amino acids. Polypeptides may have a defined three-dimensional structure, although they do not necessarily have such structure. Polypeptides with a defined three-dimensional structure are referred to as folded, and polypeptides which do not possess a defined three-dimensional structure, but rather can adopt a large number of different conformations, and are referred to as unfolded. As used herein, the term glycoprotein refers to a protein coupled to at least one carbohydrate moiety that is attached to the protein via an oxygen-containing or a nitrogen-containing side chain of an amino acid residue, *e.g.*, a serine residue or an asparagine residue.

[0047] By an "isolated" polypeptide or a fragment, variant, or derivative thereof is intended a polypeptide that is not in its natural milieu. No particular level of purification is required. For example, an isolated polypeptide can be removed from its native or natural environment. Recombinantly produced polypeptides and proteins expressed in host cells are considered isolated for purposed of the invention, as are native or recombinant polypeptides which have been separated, fractionated, or partially or substantially purified by any suitable technique.

[0048] Also included as polypeptides of the present invention are fragments, derivatives, analogs, or variants of the foregoing polypeptides, and any combination thereof. The terms "fragment," "variant," "derivative" and "analog" when referring to antibodies or antibody polypeptides of the present invention include any polypeptides which retain at least some of the antigen-binding properties of the corresponding native binding molecule, antibody, or polypeptide. Fragments of polypeptides of the present invention include proteolytic fragments, as well as deletion fragments, in addition to specific antibody fragments discussed elsewhere herein. Variants of antibodies and antibody polypeptides of the present invention include fragments as described above, and also polypeptides with altered

amino acid sequences due to amino acid substitutions, deletions, or insertions. Variants may occur naturally or be non-naturally occurring Non-naturally occurring variants may be produced using art-known mutagenesis techniques. Variant polypeptides may comprise conservative or non-conservative amino acid substitutions, deletions or additions. Derivatives of neoepitope-specific binding molecules, *e.g.*, antibodies and antibody polypeptides of the present invention, are polypeptides which have been altered so as to exhibit additional features not found on the native polypeptide. Examples include fusion proteins. Variant polypeptides may also be referred to herein as "polypeptide analogs." As used herein a "derivative" of a binding molecule or fragment thereof, an antibody, or an antibody polypeptide refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Also included as "derivatives" are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For example, 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine.

[0049] The term "polynucleotide" is intended to encompass a singular nucleic acid as well as plural nucleic acids, and refers to an isolated nucleic acid molecule or construct, *e.g.*, messenger RNA (mRNA) or plasmid DNA (pDNA). A polynucleotide may comprise a conventional phosphodiester bond or a non-conventional bond (*e.g.*, an amide bond, such as found in peptide nucleic acids (PNA)). The term "nucleic acid" refer to any one or more nucleic acid segments, *e.g.*, DNA or RNA fragments, present in a polynucleotide. By "isolated" nucleic acid or polynucleotide is intended a nucleic acid molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding an antibody contained in a vector is considered isolated for the purposes of the present invention. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of polynucleotides of the present invention. Isolated polynucleotides or nucleic acids according to the present invention further include such molecules produced synthetically. In addition, polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

[0050] As used herein, a "coding region" is a portion of nucleic acid which consists of codons translated into amino acids. Although a "stop codon" (TAG, TGA, or TAA) is not translated into an amino acid, it may be considered to be part of a coding region, but any flanking sequences, for example promoters, ribosome binding sites, transcriptional terminators, introns, and the like, are not part of a coding region. Two or more coding regions of the present invention can be present in a single polynucleotide construct, *e.g.*, on a single vector, or in separate polynucleotide constructs, *e.g.*, on separate (different) vectors. Furthermore, any vector may contain a single coding region, or may comprise two or more coding regions, *e.g.*, a single vector may separately encode an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region. In addition, a vector, polynucleotide, or nucleic acid of the invention may encode heterologous coding regions, either fused or unfused to a nucleic acid encoding a binding molecule, an antibody, or fragment, variant, or derivative thereof. Heterologous coding regions include without limitation specialized elements or motifs, such as a secretory signal peptide or a heterologous functional domain.

[0051] In certain embodiments, the polynucleotide or nucleic acid is DNA. In the case of DNA, a polynucleotide comprising a nucleic acid which encodes a polypeptide normally may include a promoter and/or other transcription or translation control elements operably associated with one or more coding regions. An operable association is when a coding region for a gene product, *e.g.*, a polypeptide, is associated with one or more regulatory sequences in such a way as to place expression of the gene product under the influence or control of the regulatory sequence(s). Two DNA fragments (such as a polypeptide coding region and a promoter associated therewith) are "operably associated" if induction of promoter function results in the transcription of mRNA encoding the desired gene product and if the nature of the linkage between the two DNA fragments does not interfere with the ability of the expression regulatory sequences to direct the expression of the gene product or interfere with the ability of the DNA template to be transcribed. Thus, a promoter region would be operably associated with a nucleic acid encoding a polypeptide if the promoter was capable of effecting transcription of that nucleic acid. The promoter may be a cell-specific promoter that directs substantial transcription of the DNA only in predetermined cells. Other transcription control elements, besides a promoter, for example enhancers, operators, repressors, and transcription termination signals, can be operably

associated with the polynucleotide to direct cell-specific transcription. Suitable promoters and other transcription control regions are disclosed herein.

[0052] A variety of transcription control regions are known to those skilled in the art. These include, without limitation, transcription control regions which function in vertebrate cells, such as, but not limited to, promoter and enhancer segments from cytomegaloviruses (the immediate early promoter, in conjunction with intron-A), simian virus 40 (the early promoter), and retroviruses (such as Rous sarcoma virus). Other transcription control regions include those derived from vertebrate genes such as actin, heat shock protein, bovine growth hormone and rabbit ß-globin, as well as other sequences capable of controlling gene expression in eukaryotic cells. Additional suitable transcription control regions include tissue-specific promoters and enhancers as well as lymphokine-inducible promoters (*e.g.*, promoters inducible by interferons or interleukins).

[0053] Similarly, a variety of translation control elements are known to those of ordinary skill in the art. These include, but are not limited to ribosome binding sites, translation initiation and termination codons, and elements derived from picornaviruses (particularly an internal ribosome entry site, or IRES, also referred to as a CITE sequence).

[0054] In other embodiments, a polynucleotide of the present invention is RNA, for example, in the form of messenger RNA (mRNA).

[0055] Polynucleotide and nucleic acid coding regions of the present invention may be associated with additional coding regions which encode secretory or signal peptides, which direct the secretion of a polypeptide encoded by a polynucleotide of the present invention. According to the signal hypothesis, proteins secreted by mammalian cells have a signal peptide or secretory leader sequence which is cleaved from the mature protein once export of the growing protein chain across the rough endoplasmic reticulum has been initiated. Those of ordinary skill in the art are aware that polypeptides secreted by vertebrate cells generally have a signal peptide fused to the N-terminus of the polypeptide, which is cleaved from the complete or "full length" polypeptide to produce a secreted or "mature" form of the polypeptide. In certain embodiments, the native signal peptide, *e.g.*, an immunoglobulin heavy chain or light chain signal peptide is used, or a functional derivative of that sequence that retains the ability to direct the secretion of the polypeptide that is operably associated with it. Alternatively, a heterologous mammalian signal peptide, or a functional derivative thereof, may be used. For example, the wild-type leader sequence

may be substituted with the leader sequence of human tissue plasminogen activator (TPA) or mouse ß-glucuronidase.

[0056] Unless stated otherwise, the terms "disorder" and "disease" are used interchangeably herein. A "binding molecule" as used in the context of the present invention relates primarily to antibodies, and fragments thereof, but may also refer to other non-antibody molecules that bind to a neoepitope including but not limited to hormones, receptors, ligands, major histocompatibility complex (MHC) molecules, chaperones such as heat shock proteins (HSPs) as well as cell-cell adhesion molecules such as members of the cadherin, intergrin, C-type lectin and immunoglobulin (Ig) superfamilies. Thus, for the sake of clarity only and without restricting the scope of the present invention most of the following embodiments are discussed with respect to antibodies and antibody-like molecules which represent the preferred binding molecules for the development of therapeutic and diagnostic agents.

[0057] The terms "antibody" and "immunoglobulin" are used interchangeably herein. An antibody or immunoglobulin is an antigen-binding molecule which comprises at least the variable domain of a heavy chain, and normally comprises at least the variable domains of a heavy chain and a light chain. Basic immunoglobulin structures in vertebrate systems are relatively well understood. See, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988).

[0058] As will be discussed in more detail below, the term "immunoglobulin" comprises various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, ε) with some subclasses among them (*e.g.*, γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention. All immunoglobulin classes are clearly within the scope of the present invention, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately

23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

[0059] Light chains are classified as either kappa or lambda (κ, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0060] Both the light and heavy chains are divided into regions of structural and functional homology. The terms "constant" and "variable" are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CL domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0061] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen binding site. This quaternary antibody structure forms the antigen binding site present at the end of each arm of the Y. More specifically, the antigen binding site is defined by three CDRs on each of the VH and VL chains. Any antibody or immunoglobulin fragment which contains sufficient structure to specifically bind to an antigen is denoted herein interchangeably as an "antigen binding fragment" or an "immunospecific fragment."

[0062] In naturally occurring antibodies, the six "complementarity determining regions" or "CDRs" present in each antigen binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen binding domains, referred to as "framework" regions, show less inter-molecular variability. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (see, "Sequences of Proteins of Immunological Interest," Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol., 196*:901-917 (1987), which are incorporated herein by reference in their entireties).

[0063] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term "complementarity determining region" ("CDR") to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia *et al.*, *J. Mol. Biol. 196*:901-917 (1987), which are incorporated herein by reference, where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth below in Table I as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely

determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

Table 1: CDR Definitions[1]

	Kabat	Chothia
VH CDR1	31-35	26-32
VH CDR2	50-65	52-58
VH CDR3	95-102	95-102
VL CDR1	24-34	26-32
VL CDR2	50-56	50-52
VL CDR3	89-97	91-96

[1]Numbering of all CDR definitions in Table 1 is according to the numbering conventions set forth by Kabat *et al.* (see below).

[0064] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in an antibody or antigen-binding fragment, variant, or derivative thereof of the present invention are according to the Kabat numbering system.

[0065] Antibodies or antigen-binding fragments, immunospecific fragments, variants, or derivatives thereof of the invention include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and $F(ab')_2$, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VL or VH domain, fragments produced by a Fab expression library, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies disclosed herein). ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019. Immunoglobulin or antibody molecules of the invention can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0066] In one embodiment, the antibody of the present invention is not IgM or a derivative thereof with a pentavalent structure. Particular, in specific applications of the present invention, especially therapeutic use, IgMs are less useful than IgG and other bivalent antibodies or corresponding binding molecules since IgMs due to their pentavalent structure and lack of affinity maturation often show unspecific cross-reactivities and very low affinity.

[0067] Antibody fragments, including single-chain antibodies, may comprise the variable region(s) alone or in combination with the entirety or a portion of the following: hinge region, CH1, CH2, and CH3 domains. Also included in the invention are antigen-binding fragments also comprising any combination of variable region(s) with a hinge region, CH1, CH2, and CH3 domains. Antibodies or immunospecific fragments thereof of the present invention may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condricthoid in origin (*e.g.*, from sharks). As used herein, "human" antibodies include antibodies having the amino acid sequence of a human immunoglobulin and include antibodies isolated from human patients, human immunoglobulin libraries or from animals transgenic for one or more human immunoglobulins and that do not express endogenous immunoglobulins, as described *infra* and, for example in, U.S. Pat. No. 5,939,598 by Kucherlapati *et al.* A human antibody is still "human" even if amino acid substitutions are made in the antibody, *e.g.*, to improve binding characteristics.

[0068] As used herein, the term "heavy chain portion" includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain portion comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, a binding polypeptide for use in the invention may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the invention comprises a polypeptide chain comprising a CH3 domain. Further, a binding polypeptide for use in the invention may lack at least a

portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that these domains (*e.g.*, the heavy chain portions) may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0069] In certain antibodies, or antigen-binding fragments, variants, or derivatives thereof disclosed herein, the heavy chain portions of one polypeptide chain of a multimer are identical to those on a second polypeptide chain of the multimer. Alternatively, heavy chain portion-containing monomers of the invention are not identical. For example, each monomer may comprise a different target binding site, forming, for example, a bispecific antibody.

[0070] The heavy chain portions of a binding polypeptide for use in the diagnostic and treatment methods disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain portion of a polypeptide may comprise a CH1 domain derived from an IgG1 molecule and a hinge region derived from an IgG3 molecule. In another example, a heavy chain portion can comprise a hinge region derived, in part, from an IgG1 molecule and, in part, from an IgG3 molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG1 molecule and, in part, from an IgG4 molecule.

[0071] As used herein, the term "light chain portion" includes amino acid sequences derived from an immunoglobulin light chain. Preferably, the light chain portion comprises at least one of a VL or CL domain.

[0072] The minimum size of a peptide or polypeptide epitope for an antibody is thought to be about four to five amino acids. Peptide or polypeptide epitopes preferably contain at least seven, more preferably at least nine and most preferably between at least about 15 to about 30 amino acids. Since a CDR can recognize an antigenic peptide or polypeptide in its tertiary form, the amino acids comprising an epitope need not be contiguous, and in some cases, may not even be on the same peptide chain. In the present invention, peptide or polypeptide epitope recognized by antibodies of the present invention contains a sequence of at least 4, at least 5, at least 6, at least 7, more preferably at least 8, at least 9, at least 10, at least 15, at least 20, at least 25, or between about 15 to about 30 contiguous or non-contiguous amino acids of Aβ.

[0073] The term "neoepitope" in accordance with the present invention denotes an epitope which is unique for a disease pattern and contained in or formed by a disorder-associated

protein which is a pathological variant from an otherwise non-pathological protein and/or deviating from the physiology of the healthy state. Said pathophysiological variants can be formed by means of pathologically altered transcription, pathologically altered translation, post-translational modification, pathologically altered proteolytic processing, pathologically altered complex formation with physiological or pathophysiological interaction partners or cellular structures in the sense of an altered co-localization, or pathologically altered structural conformation – like for example aggregation, oligomerization or fibrillation – whose three- or four-dimensional structure differs from the structure of the physiologically active molecule. Moreover, a pathophysiological variant can also be characterized in that it is not located in its usual physiological environment or subcellular compartment. As an example, neoepitopes may be located in the pathologically conspicuous structures in the areas of brain tissues that obviously experience or have already experienced functional damage. Whether a given structure, for example cell or tissue, or protein displays a neoepitope can be verified by reversing the method described below for isolating and characterizing a disorder-associated protein specific binding molecule in that a binding molecule, for example antibody identified by said method is used to screen a sample for binding to the antibody, thereby determining the presence of a neoepitope.

[0074] The phrases "disease-associated protein specific" and "neoepitope specific" are used interchangeably herein with the term "specifically recognizing a neoepitope". As used herein terms such as "absence of cross-reactivity", "specific," " specifically recognizing," "specifically binding," "preferentially binding," and the like refer to the binding molecule's ability to discriminate between the neoepitope of a disorder-associated protein and the native protein in its wild type form and natural context. Thus, the binding molecule of the present invention has a preferential binding affinity to the neoepitope over the native protein antigen by a factor of at least two, preferably at least 5, usually more than by a factor of 10, particularly preferred by a factor of 50 and even more preferred higher than 100. Furthermore, the relative K_D of the binding molecule, *e.g.*, antibody for the specific target epitope, *e.g.* neoepitope is preferably at least 10-fold less, more preferably at least 100-fold less or more than the K_D for binding that antibody to other ligands or to the native counterpart of the disease-associated protein.

[0075] By "specifically binds," or "specifically recognizes," used interchangeably herein, it is generally meant that a binding molecule, *e.g.*, an antibody binds to an epitope via its

antigen binding domain, and that the binding entails some complementarity between the antigen binding domain and the epitope. According to this definition, an antibody is said to "specifically bind" to an epitope when it binds to that epitope, via its antigen binding domain more readily than it would bind to a random, unrelated epitope. The term "specificity" is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody "A" may be deemed to have a higher specificity for a given epitope than antibody "B," or antibody "A" may be said to bind to epitope "C" with a higher specificity than it has for related epitope "D."

[0076] By "preferentially binds," it is meant that the binding molecule, *e.g.*, antibody specifically binds to an epitope more readily than it would bind to a related, similar, homologous, or analogous epitope. Thus, an antibody which "preferentially binds" to a given epitope would more likely bind to that epitope than to a related epitope, even though such an antibody may cross-react with the related epitope.

[0077] By way of non-limiting example, a binding molecule, *e.g.*, an antibody may be considered to bind a first epitope preferentially if it binds said first epitope with a dissociation constant (K_D) that is less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first antigen preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's K_D for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's K_D for the second epitope.

[0078] In another non-limiting example, a binding molecule, *e.g.*, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an off rate (k(off)) that is less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least one order of magnitude less than the antibody's k(off) for the second epitope. In another non-limiting example, an antibody may be considered to bind a first epitope preferentially if it binds the first epitope with an affinity that is at least two orders of magnitude less than the antibody's k(off) for the second epitope.

[0079] A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a target polypeptide disclosed herein or a

fragment or variant thereof with an off rate (k(off)) of less than or equal to 5 X 10^{-2} sec^{-1}, 10^{-2} sec^{-1}, 5 X 10^{-3} sec^{-1} or 10^{-3} sec^{-1}. More preferably, an antibody of the invention may be said to bind a target polypeptide disclosed herein or a fragment or variant thereof with an off rate (k(off)) less than or equal to 5 X 10^{-4} sec^{-1}, 10^{-4} sec^{-1}, 5 X 10^{-5} sec^{-1}, or 10^{-5} sec^{-1} 5 X 10^{-6} sec^{-1}, 10^{-6} sec^{-1}, 5 X 10^{-7} sec^{-1} or 10^{-7} sec^{-1}.

[0080] A binding molecule, *e.g.*, an antibody or antigen-binding fragment, variant, or derivative disclosed herein may be said to bind a target polypeptide disclosed herein or a fragment or variant thereof with an on rate (k(on)) of greater than or equal to 10^3 M^{-1} sec^{-1}, 5 X 10^3 M^{-1} sec^{-1}, 10^4 M^{-1} sec^{-1} or 5 X 10^4 M^{-1} sec^{-1}. More preferably, an antibody of the invention may be said to bind a target polypeptide disclosed herein or a fragment or variant thereof with an on rate (k(on)) greater than or equal to 10^5 M^{-1} sec^{-1}, 5 X 10^5 M^{-1} sec^{-1}, 10^6 M^{-1} sec^{-1}, or 5 X 10^6 M^{-1} sec^{-1} or 10^7 M^{-1} sec^{-1}.

[0081] A binding molecule, *e.g.*, an antibody is said to competitively inhibit binding of a reference antibody to a given epitope if it preferentially binds to that epitope to the extent that it blocks, to some degree, binding of the reference antibody to the epitope. Competitive inhibition may be determined by any method known in the art, for example, competition ELISA assays. An antibody may be said to competitively inhibit binding of the reference antibody to a given epitope by at least 90%, at least 80%, at least 70%, at least 60%, or at least 50%.

[0082] As used herein, the term "affinity" refers to a measure of the strength of the binding of an individual epitope with the CDR of a binding molecule, *e.g.*, an immunoglobulin molecule. *See*, *e.g.*, Harlow *et al.*, Antibodies: A Laboratory Manual, (Cold Spring Harbor Laboratory Press, 2nd ed. 1988) at pages 27-28. As used herein, the term "avidity" refers to the overall stability of the complex between a population of immunoglobulins and an antigen, that is, the functional combining strength of an immunoglobulin mixture with the antigen. *See*, *e.g.*, Harlow at pages 29-34. Avidity is related to both the affinity of individual immunoglobulin molecules in the population with specific epitopes, and also the valencies of the immunoglobulins and the antigen. For example, the interaction between a bivalent monoclonal antibody and an antigen with a highly repeating epitope structure, such as a polymer, would be one of high avidity.

[0083] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof of the invention may also be described or specified in terms of their cross-reactivity. As used herein, the term "cross-reactivity" refers to the ability of an

antibody, specific for one antigen, to react with a second antigen; a measure of relatedness between two different antigenic substances. Thus, an antibody is cross reactive if it binds to an epitope other than the one that induced its formation. The cross reactive epitope generally contains many of the same complementary structural features as the inducing epitope, and in some cases, may actually fit better than the original.

[0084] For example, certain antibodies have some degree of cross-reactivity, in that they bind related, but non-identical epitopes, *e.g.*, epitopes with at least 95%, at least 90%, at least 85%, at least 80%, at least 75%, at least 70%, at least 65%, at least 60%, at least 55%, and at least 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be said to have little or no cross-reactivity if it does not bind epitopes with less than 95%, less than 90%, less than 85%, less than 80%, less than 75%, less than 70%, less than 65%, less than 60%, less than 55%, and less than 50% identity (as calculated using methods known in the art and described herein) to a reference epitope. An antibody may be deemed "highly specific" for a certain epitope, if it does not bind any other analog, ortholog, or homolog of that epitope.

[0085] Binding molecules, *e.g.*, antibodies or antigen-binding fragments, variants or derivatives thereof of the invention may also be described or specified in terms of their binding affinity to a polypeptide of the invention. Preferred binding affinities include those with a dissociation constant or Kd less than 5×10^{-2} M, 10^{-2} M, 5×10^{-3} M, 10^{-3} M, 5×10^{-4} M, 10^{-4} M, 5×10^{-5} M, 10^{-5} M, 5×10^{-6} M, 10^{-6} M, 5×10^{-7} M, 10^{-7} M, 5×10^{-8} M, 10^{-8} M, 5×10^{-9} M, 10^{-9} M, 5×10^{-10} M, 10^{-10} M, 5×10^{-11} M, 10^{-11} M, 5×10^{-12} M, 10^{-12} M, 5×10^{-13} M, 10^{-13} M, 5×10^{-14} M, 10^{-14} M, 5×10^{-15} M, or 10^{-15} M.

[0086] As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term "VH domain" includes the amino terminal variable domain of an immunoglobulin heavy chain and the term "CH1 domain" includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0087] As used herein the term "CH2 domain" includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; see Kabat EA *et al. op. cit.* The CH2 domain is

unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0088] As used herein, the term "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol. 161*:4083 (1998)).

[0089] As used herein the term "disulfide bond" includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CL regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0090] As used herein, the term "engineered antibody" refers to an antibody in which the variable domain in either the heavy and light chain or both is altered by at least partial replacement of one or more CDRs from an antibody of known specificity and, if necessary, by partial framework region replacement and sequence changing. Although the CDRs may be derived from an antibody of the same class or even subclass as the antibody from which the framework regions are derived, it is envisaged that the CDRs will be derived from an antibody of different class and preferably from an antibody from a different species. An engineered antibody in which one or more "donor" CDRs from a non-human antibody of known specificity is grafted into a human heavy or light chain framework region is referred to herein as a "humanized antibody." It may not be necessary to replace all of the CDRs with the complete CDRs from the donor variable region to transfer the antigen binding capacity of one variable domain to another. Rather, it may only be necessary to transfer those residues that are necessary to maintain the activity of the target binding site. Given the explanations set forth in, *e.g.*, U. S. Pat. Nos. 5,585,089, 5,693,761, 5,693,762, and 6,180,370, it will be well within the competence of those skilled in the art, either by carrying out routine experimentation or by trial and error testing to obtain a functional engineered or humanized antibody.

[0091] As used herein the term "properly folded polypeptide" includes polypeptides in which all of the functional domains comprising the polypeptide are distinctly active. As used herein, the term "improperly folded polypeptide" includes polypeptides in which at least one of the functional domains of the polypeptide is not active. In one embodiment, a properly folded polypeptide comprises polypeptide chains linked by at least one disulfide bond and, conversely, an improperly folded polypeptide comprises polypeptide chains not linked by at least one disulfide bond.

[0092] As used herein the term "engineered" includes manipulation of nucleic acid or polypeptide molecules by synthetic means (*e.g.* by recombinant techniques, *in vitro* peptide synthesis, by enzymatic or chemical coupling of peptides or some combination of these techniques).

[0093] As used herein, the terms "linked," "fused" or "fusion" are used interchangeably. These terms refer to the joining together of two more elements or components, by whatever means including chemical conjugation or recombinant means. An "in-frame fusion" refers to the joining of two or more polynucleotide open reading frames (ORFs) to form a continuous longer ORF, in a manner that maintains the correct translational reading frame of the original ORFs. Thus, a recombinant fusion protein is a single protein containing two ore more segments that correspond to polypeptides encoded by the original ORFs (which segments are not normally so joined in nature.) Although the reading frame is thus made continuous throughout the fused segments, the segments may be physically or spatially separated by, for example, in-frame linker sequence. For example, polynucleotides encoding the CDRs of an immunoglobulin variable region may be fused, in-frame, but be separated by a polynucleotide encoding at least one immunoglobulin framework region or additional CDR regions, as long as the "fused" CDRs are co-translated as part of a continuous polypeptide.

[0094] In the context of polypeptides, a "linear sequence" or a "sequence" is an order of amino acids in a polypeptide in an amino to carboxyl terminal direction in which residues that neighbor each other in the sequence are contiguous in the primary structure of the polypeptide.

[0095] The term "expression" as used herein refers to a process by which a gene produces a biochemical, for example, an RNA or polypeptide. The process includes any manifestation of the functional presence of the gene within the cell including, without limitation, gene knockdown as well as both transient expression and stable expression. It includes without

limitation transcription of the gene into messenger RNA (mRNA), transfer RNA (tRNA), small hairpin RNA (shRNA), small interfering RNA (siRNA) or any other RNA product, and the translation of such mRNA into polypeptide(s). If the final desired product is a biochemical, expression includes the creation of that biochemical and any precursors. Expression of a gene produces a "gene product." As used herein, a gene product can be either a nucleic acid, *e.g.*, a messenger RNA produced by transcription of a gene, or a polypeptide which is translated from a transcript. Gene products described herein further include nucleic acids with post transcriptional modifications, *e.g.*, polyadenylation, or polypeptides with post translational modifications, *e.g.*, methylation, glycosylation, the addition of lipids, association with other protein subunits, proteolytic cleavage, and the like.

[0096] As used herein, the terms "treat" or "treatment" refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. "Treatment" can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the manifestation of the condition or disorder is to be prevented.

[0097] By "subject" or "individual" or "animal" or "patient" or „mammal," is meant any subject, particularly a mammalian subject, *e.g.*, a human patient, for whom diagnosis, prognosis, prevention, or therapy is desired.

II. Methods to Identify Binding Molecules

[0098] The present invention generally relates to means and methods for discovering therapeutically efficient antibodies from clinically preselected human subjects. As demonstrated in the examples, human antibodies against abnormal structures in human brain diseases can be isolated from phenotypically healthy, or clinically unusually stable patients and corresponding recombinant antibodies can be successfully used for the treatment, amelioration of pathology and prevention of impairment of brain function

without substantial side effects. Clinical stability or non-progression of disease can be identified by way of example by measurement over time of clinical, e.g., cognitive, status (by neuropsychological testing, for example); assessment of the global functional level; evaluation of the daily living capacities or behavioral deficits; volumetric analysis of brain structures; *in vivo* measurement of pathological deposits of abnormal proteins in brain (*e.g.* PET beta-amyloid imaging) or biochemical variables in body fluids (*e.g.* tau proteins, or Abeta peptides); and by comparison to the natural course/history of the disease. Thus, the present invention provides antibodies and binding molecules which are capable of recognizing neoepitopes of disease-associated proteins which derive from native endogenous proteins, and are prevalent in the body of a patient in a variant form, *e.g.* as a pathological protein and/or out of their normal physiological context. In particular, antibodies and antigen-binding fragments thereof are provided, which demonstrate the immunological binding characteristics and/or biological properties as outlined for the antibody illustrated in the Examples. Where present, the term "immunological binding characteristics," or other binding characteristics of an antibody with an antigen, in all of its grammatical forms, refers to the specificity, affinity, cross-reactivity, and other binding characteristics of an antibody. Naturally, the present invention extends to the antibody producing cell lines and recombinant cells as well. The present invention further relates to diagnostic assays and kits that comprise the binding molecule of the present invention and to therapeutic methods and therapeutic evaluations based thereon.

[0099] The present invention is based on the observation that in human subjects and patients preselected according to specific clinical criteria, who bear a risk of developing a neurological disorder like Alzheimer's disease due to their age, as related to the humoral defense, antibodies to endogenous pathophysiological variants like Abeta peptide aggregates, neurofibrillary tangles, dystrophic neurites and further cellular structures can also be found, which are neuropathologically characteristic for the disorder. These structures can be found alone or in combination with other pathological structures and can, as in case of the Abeta aggregates and the precursors of the neurofibrillary tangles, develop pathological effects. However, those antibodies specifically recognizing said structures exhibit no or significantly lower cross-reactivity to the normal physiologically functional forms of the proteins underlying the pathological structures, contrary to the known case of an autoimmune response.

[0100] Without intending to be bound by theory it is, based on the experiments performed in accordance with the present invention, believed that a disorder does not have to become manifest and phenotypically perceivable, as in case of an infection, in order to result in an experimentally measurable activity of the humoral immune system like the generation or activation of specific B cells or B memory cells. In case of tumor cells or differentiated cells, which have to be physiologically disposed of, mechanisms are already known, wherein partners like T4 helper cells and cytotoxic T cells and natural killer cells of the cellular immune system cooperate in the induction of apoptosis. It has to be assumed that, also in a healthy human, tumor cells or precursors thereof are formed on a daily basis by means of mutation. However, these are immediately driven to apoptosis by means of humoral and cellular mechanisms, so that a tumor is not detectable. In this sense, a "healthy, or clinically unusually stable, patient" is not understood to denote an individual in whom no disease events take place, but an individual in whom the diverse disease events are controlled by blocking or defense mechanisms before they phenotypically manifest themselves.

[0101] In view of the above, it was hypothesized in accordance with the present invention that it should be possible to identify an antibody or an antibody-producing cell from specific patient collectives or healthy subjects, which have been preselected according to clinical criteria, without precognition of the molecular nature of a target structure epitope, *e.g.* neoepitope, wherein the antibody, in case of already prevailing tolerance in the donor organism – which is, for example, proved by the absence of autoimmunity – could successfully be employed against a disease in form of a recombinantly produced agent. Identifying such an antibody can thus be conducted without precognition of the molecular epitope of the target structure, but rather solely by means of binding to neoepitopes of pathologically conspicuous structures in clinico-pathologically well characterized tissue sections derived from human patients or from animal models of the respective disease.

[0102] Accordingly, in a first aspect the present invention relates to a method of isolating a disorder-associated protein-specific binding molecule comprising:

[0103] (a) subjecting a sample obtained from a patient who is symptom-free, or who is clinically unusually stable, but who is affected with or at risk of developing a disorder or effectively suppressing the manifestation or outbreak of a disorder to a specimen of pathologically or physiologically altered cells or tissue of predetermined clinical characteristics; and

[0104] (b) identifying and optionally isolating a binding molecule which preferentially binds to said specimen but not or with significantly lower affinity to corresponding cells or tissues without such pathological characteristics as it may be derived from a healthy subject.

[0105] The method of the present invention can be performed as outlined in the Examples section with means well known to a person skilled in the art. For example, a liquid sample obtained from the patient can be passed through a first aperture of a duct which is in contact with the specimen target structure firmly held in an object holder, thereby allowing putative binding molecules present in the sample, either in a soluble form or expressed on the cell surface and membrane, respectively, to bind to said target structure. The liquid sample may contain for example lymphocytes and/or antibodies while the specimen may be a tissue section or a membrane coated with molecules or molecular combinations which are distinct for a pathological target structure.

[0106] Any non-binding matter can be removed via the second duct aperture. At the same time, the temperature of the object holder may be controlled by an object holder thermostat, for example at a temperature at which natural binding of the putative binding molecule to the neoepitope of the antigen specific for the specimen takes place in the human body. By way of the flowing motion, *e.g.* passing the liquid sample containing binding molecules, preferably at body temperature over the target structure natural systems of binding interactions can be simulated. However, other methods of incubating the sample with the specimen such as by means of a shaker or rotating table may be used as well. A particular advantage of the above-mentioned system is that it allows an interruption of metabolic processes at any time by decreasing the temperature of the object holder by means of the object holder thermostat. In doing so, the temperature of the object holder can be decreased to for example 2-10°C, in particular 4°C. A corresponding device that can be used in accordance with the method of the present invention is described in European patent application EP 1 069 431 A2. Hence, the method of the present invention will allow identification and characterization of the binding partners as well as at the same time to identify and characterize the molecular classes, molecular groups and/or molecular parts required for the binding process, *i.e.* the target structures of the specimen, which hitherto may be unknown. This will not only open up new possible ways of diagnosis, but will also provide a new test system for therapeutic approaches on a molecular level.

[0107] As a patient may qualify in accordance with the present invention a pool of healthy volunteers if specific surrogate markers predict a high probability of a status of a disease, which has surprisingly – and possibly due to a specific endogenous immune response – not become clinically manifest, however. In this sense, as related to diverse diseases, a healthy elderly human would be an individual, in which a neurodegenerative disease like Alzheimer's disease or Parkinson's disease is not yet clinically manifest, but in which the preclinical development of pathophysiological protein variants, *i.e.* disorder-associated proteins, and in the above-mentioned sense by means of early intervention of the humoral immune system with or without involvement of cellular components of the immune system, has been restricted or delayed to such an extent that, until the moment when the healthy, but not preclinical patient participated in the study, the clinical manifestation of the disease had not yet occurred. Preferably, inconspicuous volunteers in whom neither autoimmunological processes have been diagnosed nor other possible pathological conditions occur as side effects are recruited as donors for the sample in the first instance.

[0108] In principle samples from patients may be used, who have undergone an active immunization with variants of physiological proteins or peptides, wherein the antibody development has been boosted by the immunization. Antibodies, for example, can be identified and isolated from Alzheimer's disease patients that have been vaccinated with Abeta peptide. *See for e.g.*, Hock *et al., Nature Medicine 8*:1280-1275 (2002), Hock *et al.*, *Neuron 38*:547-554 (2003), and WO 2004/095031, each incorporated herein by reference in their entireties. However, it may be preferred to use samples from volunteers which have not received such immunization or corresponding medication concerning the disorder the variant pathological protein is associated with and/or causative of.

[0109] According to the present invention, samples of a patient, *e.g.* of individuals that have been clinically preselected are analyzed for the presence of binding molecules specifically recognizing specimen of pathologically conspicuous structures, for example in *ex vivo* tissue from clinico-pathologically characterized human patients or animal models like, for example, transgenic mice, or *in vitro* cell structures, or in pathological allogenic or xenogenic tissue. Preferably, said patient and/or as said subject providing the specimen are human, most preferably both.

[0110] The characteristic pathologically or physiologically altered sample, cell or tissue specimen is preferably displayed by optical detection after reaction with a binding molecule, *e.g.* antibody of the present invention. The specimen may be obtained as/from a

cell sample, tissue section, cellular smear test, cell or tissue sample of an animal model of a human disease or *in vitro* cultured cell and tissue material. Preferably, at least one of said specimen is present in an object holder in form of a scan positions, wherein each scan position corresponds to a specific pathological structure and at least two of the scan positions are concomitantly exposed to detection of a reaction with the antibody. By way of example, antibodies to the neuropathological hallmarks of such neurodegenerative diseases as Alzheimer's disease, Parkinson's disease or tauopathies – including beta-amyloid plaques, Lewy bodies, neurofibrillary tangles and dystrophic neuritis can be detected on sections obtained from human post-mortem brain tissues with clinico-pathologically confirmed diagnoses of the respective disease entities. This is done by mounting small rods of paraffin embedded tissue on to glass slides which are then probed with samples of a patient or healthy donor. The detection of a reaction with the antibody is done following standard procedures of immunohistochemistry and microscopical scanning.

[0111] Multiple tissue microsections containing various human disease tissues can be assembled on the glass slide to form a multiple tissue microarray. Similarly, additional specimens from tissue samples of animal models of human disease, cellular smear or cells can be embedded in paraffin and mounted on glass slides alone or in combination with above described human post-mortem Alzheimer's disease brain tissue or tissue arrays. Thus, a single test position on the glass slide can comprise mixed arrays of tissue and other specimen displaying pathologically conspicuous structures.

[0112] In order to increase the throughput of the assay, more than one test position is mounted on a glass slide. Preferably, eight test positions are mounted onto glass slides in a two by four format fitting the 96 well or microtiter format. A more detailed description of this microtiter-compatible tissue microarray can be found *infra* in the supplementary methods section below.

[0113] As mentioned the sample to be analyzed may comprise a body fluid, a cell sample or the supernatant of a cell sample or a derivative thereof. Body fluids such as lumbar cerebrospinal fluid (CSF), plasma or urine can be collected following standard clinical procedures after informed consent of the patients. Most preferably, the sample comprises or is derived from B-cells or memory B-cells and/or comprises antibodies.

[0114] Preferably, said patient has been determined to be affected with a not yet manifested disorder or at risk to develop the disorder by the presence or absence of a surrogate marker,

or by an unusually stable disease course. Clinical criteria are to be considered in connection with surrogate markers having either an increased probability of the occurrence or the manifestation of a disease according to the present invention in the sense of a preclinical condition, or, vice versa, proving the improbability of such a disease already having occurred as, for example, a genetic constellation promoting the disease is existent or an extreme exposition or way of living renders the phenotypical development of a disease probable. In the case of Alzheimer's disease this means that, according to the present invention, such human volunteers are searched for B cells or memory B cells against neuropathology-associated protein complexes like Abeta aggregates, oligomeric Abeta species and beta-amyloid fibrils in beta-amyloid plaques, for tau filaments in neurofibrillary tangles, for alpha synuclein in Lewy bodies or components hitherto not molecularly identified that, with respect to age, belong to a group of individuals in whom either the prevalence of Alzheimer's disease is particularly high or who originate genetically from a population bearing a high risk for Alzheimer's disease. These are, for example, persons older than 75 years having no or only marginal neuropsychologically measurable cognitive impairments, or in case of tumor indications, persons having highly indicative tumor markers, for example genetic tumor markers, but not suffering from the disease (Alloul *et al.*, Arch. Gerontology Geriatrics 27 (1998), 189; Dunn *et al.*, Immunity 21 (2004) 137-148). In case of mild cognitive impairment, these are patients that have remained clinically, neuropsychologically, or cognitively stable for years in spite of their annual statistical risk of more than 20% for developing a neurodegenerative disease that is clinically manifest and progressive in the further course. Thus, in one embodiment said surrogate marker is selected from the group consisting of old age, brain amyloid load, ApoE genotype, APP genotype, PS1 genotype, levels in body fluids of Abeta peptide, isoprostanes, Tau, and phospho-Tau.

[0115] A particular approach in employing the method according to the present invention is testing samples of B cells and B memory cells from clinically preselected volunteers against arrays of specimen of pathologically conspicuous tissues of entirely different or related primary diseases. In particular, such pathological tissues originate from human patients suffering from neurodegenerative diseases, protein-misfolding diseases, tissue amyloidoses and other diseases related to pathological deposits, autoimmune disorders, inflammatory diseases, hyperproliferative and neoplastic diseases, *e.g.*, tumors, storage

diseases and inclusion diseases as well as from animal models of human diseases, in particular from mice altered with pathologically relevant human genes.

[0116] In one particularly preferred embodiment, the present invention focuses on Alzheimer's disease. In this embodiment, the sample is obtained from a subject preferably fulfilling the following criteria:

a) being 65, preferably 70 and more preferably 75 years of age or older;

b) having full cognitive capacity and good health; and

c) having no clinical signs of dementia; or

d) having unusually slow rates of progression of disease despite the presence of an established clinical diagnosis of probable Alzheimer's disease; or

e) having unusually low conversion rates from Mild Cognitive Impairment (MCI) to full blown Alzheimer's disease.

[0117] In a further embodiment, the method of the present invention further comprises the steps of:

a) purifying B cells or B memory cells from a sample which has been identified to contain binding molecules, *e.g.* antibodies which preferentially bind to said specimen but not or with significantly lower affinity to corresponding cells or tissue of a healthy subject;

b) obtaining the immunoglobulin gene repertoire encoding said antibodies from said B cells or B memory cells; and

c) using said repertoire to express said antibodies, and optionally wherein step (b) comprises the steps of:

d) obtaining mRNA from said B cells or memory B cells;

e) obtaining cDNA from the mRNA of step (iv); and

f) using a primer extension reaction to amplify from said cDNA the fragments corresponding to the heavy chains (HC) and the kappa/lambda light chains (LC) of said antibodies.

[0118] Methods of producing clones of an immortalized human B cell and B memory lymphocyte, comprising the step of transforming human B memory lymphocytes using Epstein Barr Virus (EBV) in the presence of a polyclonal B cell activator are summarized in international application WO2004/076677. This international application also describes methods for obtaining a nucleic acid sequence that encodes an antibody of interest, comprising the steps of preparing an immortalized B cell clone and obtaining/sequencing nucleic acid from the B cell clone that encodes the antibody of interest and further inserting the nucleic acid into or using the nucleic acid to prepare an expression host that can express the antibody of interest, culturing or sub-culturing the expression host under conditions where the antibody of interest is expressed and, optionally, purifying the antibody of interest. It goes without saying that the nucleic acid may be manipulated in between to introduce restriction sites, to change codon usage, and/or to add or optimize transcription and/or translation regulatory sequences. For example, nucleic acid sequences can be generated by back-translation of the polypeptide sequences of the present invention using software such as vector NTI software to generate nucleic acid sequences that are codon-optimized and optimized for RNA stability. All this techniques are state of the art and can be performed by the person skilled in the art without undue burden. Additional methods of immortalizing human B cells are well known in the art, *e.g.*, the construction of human hybridomas or human-murine chimeric hybridomas.

[0119] In a further aspect, the present invention relates to a binding molecule which is capable of selectively recognizing an epitope of a disease-associated protein including a neoepitope of a disease-associated protein, which preferably can be obtained or validated by the method of the present invention described hereinbefore and illustrated in the examples. Advantageously, the binding molecule of the present invention does not substantially recognize said protein in its non-disorder-associated form; see also *supra*.

[0120] Means and methods for the recombinant production of binding molecules, in particular antibodies and mimics thereof as well as methods of screening for competing binding molecules, which may or may not be antibodies, are known in the art and are summarized, for example, in international application WO2006/103116 with respect to antibodies against beta-amyloid and the treatment/diagnosis of Alzheimer's disease, the disclosure content of which is incorporated herein by reference for this purpose of antibody engineering and administration for therapeutic or diagnostic applications.

[0121] However, as described herein, in particular with respect to therapeutic applications in human the antibody of the present invention is a human antibody. In this context, the variant pathological protein recognized by the antibody is preferably associated with a neurological disorder, preferably a disorder of the brain.

[0122] Moreover, as demonstrated in Examples 3 to 5 the binding molecule of the present invention, in particular an antibody has several advantageous biological properties one or more of which have been accomplished by the present invention for the first time, *e.g.* it is capable of:

(i) crossing the blood brain barrier, for example at the site of the pathological event;

(ii) binding beta-amyloid plaques, cerebrovascular amyloid, diffuse Abeta deposits, neurofibrillary tangles, hyperphosphorylated tau, alpha-synuclein positive Lewy-bodies or protein aggregates associated with dystrophic neurites;

(iii) removing beta-amyloid plaques in the brain and/or preventing the formation of amyloid plaques in the brain;

(iv) substantially restoring normal behavior; and/or

(v) causing no microhemorrhages.

[0123] In a particular preferred embodiment, the antibody or equivalent binding molecule of the present invention may be distinguished from other antibodies by one or more of the following properties, *e.g.* they are able to:

1. pass, at least in small amounts, the blood-brain barrier at the site of the pathological events;

2. bind to one or more pathophysiologically relevant extracellular or cellular structure;

3. lead to reduction of the pathophysiologically relevant structure *in vitro* or *in vivo*;

4. lead to reduction of the pathophysiologically relevant structure and to the reduction of a toxicity associated therewith;

5. lead to blocking or delaying a disease process;

6. lead to regeneration of cellular and organ-specific and organismic functions and possibly to a secondary prevention of the recurrence of the original pathophysiology after degradation of the toxicity connected with the pathophysiologically relevant structure; and/or

7. is not associated with increased microhemorrhages

[0124] Furthermore, the absence of cross-reactivity with physiological precursors or derivatives leads to the consequence that, firstly, the concentrations are predictable as *sink effects* in healthy tissue structures are avoided and, secondly, that autoimmune responses in the sense of undesired side effects are substantially missing. In addition, previous reports suggested an association of cerebral amyloid angiopathy (CAA) with compromised vascular reactivity in a transgenic mouse model with CAA (Mueggler et al., J Neurosci 22 (2002), 7218-24.). The severe CAA occurring in old arcAß mice (Knobloch et al., Neurobiol. Aging 28:1297-1306 (2007) epub July 31, 2006) might thus constrain the vasodilative flexibility of affected blood vessels. In accordance with the present invention it is prudent to expect that treatment with the antibodies of the present invention can improve vasoreactivity and cerebral blood flow in aged APP transgenic mice. This may be validated by using the arcAß mice model described in Knobloch et al. (2006), supra, and disclosed in US application "Transgenic animal model for Alzheimer's disease" by Grimm et al., serial number 60/934,291 filed on June 11, 2007, the disclosure content of which is incorporated herein by reference.

III. Antibodies

[0125] The present invention is further directed to the binding molecules *e.g.* antibodies and binding fragments, variants, and derivatives thereof shown in Table 2 and 3. The present invention is more specifically directed to an antibody, or antigen-binding fragment, variant or derivatives thereof, where the antibody specifically binds to the same neoepitope of a disorder-associated protein as a reference antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, and NI-101.13B.

[0126] The invention is further drawn to an antibody, or antigen-binding fragment, variant or derivatives thereof, where the antibody competitively inhibits a reference antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13,

NI-101.12F6A, NI-101.13A, and NI-101.13B from binding to the neoepitope of a disorder-associated protein.

[0127] The invention is also drawn to an antibody, or antigen-binding fragment, variant or derivatives thereof, where the antibody comprises an antigen binding domain identical to that of an antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, and NI-101.13B.

[0128] The present invention further exemplifies several such binding molecules, *e.g.* antibodies and binding fragments thereof, which may be characterized by comprising in their variable region, *e.g.* binding domain at least one complementarity determining region (CDR) of the V_H and/or V_L variable region comprising any one of the amino acid sequences depicted in Table 2 (V_H) and Table 3 (V_L).

Table 2: Amino acid sequences of the V_H region of neoepitope specific antibodies.

Antibody	Variable heavy chain sequence
NI-101.10	EVQLVQSGGGVVQPGRSLRLSCAASGFAFSSYGIHWVRQAPGKGLEWVAVIWFDGTKKY YTDSVKGRFTISRDNSKNTLYLQMNTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGTT VTVSS (SEQ ID NO: 4)
NI-101.11	EVQLVQSGGGVVQPGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKK YYTDSVKGRFTISRDNSKNTLYLQMNTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGT TVTVSS (SEQ ID NO: 6)
NI-101.12	EVQLVESGPGLVKPAETLSLTCTVSGGSIRSGSICWYWIRQPPGKGLEWIGYFCYSGATFY TPSLRGRLTISVDASKNQLSLSLSSVTAADTAVYYCARRAGENSGGIEPYYGMDVWGQGT TVTVSS (SEQ ID NO: 10)
NI-101.13	QVQLQESGPGLVKPSETLSLTCTVSGGSISRRSYYWGWIRQSPGKGLEWSGSIHYSGSTY YNPSLKSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARSRWGSSWVFDYWGQGTLVTVS S (SEQ ID NO: 14)
NI-101.12F 6A	QVQLVESGGGVVQPGRSLRLSCAASGFAFSSYGMHWVRQAPGKGLEWVAVIWFDGTKK YYTDSVKGRFTISRDNSKNTLYLQMNTLRAEDTAVYYCARDRGIGARRGPYYMDVWGKGT TVTVSS (SEQ ID NO: 39)
NI-101.13A	QVQLQESGPGLVKPSETLSLTCTVSGGSISRRSYYWGWIRQSPGKGLEWSGSIHYSGSTYYNPSL KSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARSRWGSSWVFDYWGQGTLVTVSS (SEQ ID NO: 42)
NI-101.13B	QVQLQESGPGLVKPSETLSLTCTVSGGSISRRSYYWGWIRQSPGKGLEWSGSIHYSGSTYYNPSL KSRVTISVDTSKNQFSLKLSSVTAADTAVYYCARSRWGSSWVFDYWGQGTLVTVSS (SEQ ID NO: 43)

Table 3: Amino acid sequences of the V_L region of neoepitope specific antibodies.

Antibody	Variable light chain sequence (kappa or lambda)

NI-101.10	EIVLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKLEIKR (SEQ ID NO: 8)
NI-101.11	EIVLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKLEIKR (SEQ ID NO: 8)
NI-101.12	DEIVLTQSPSSLSASIGDRVTITCRASESINKYVNWYQQKPGKAPKLLIYAASSLQSGAPSRV SGSGFGRDFSLTISGLQAEDFGAYFCQQSYSAPYTFGQGTKVEIKRT (SEQ ID NO: 12)
NI-101.13	QSVLTQPPSASGTPGQRVTISCSGSSSNIGSNYVYWYQQPPGTAPKLLIYRNNQRPSGVP DRFSGSKSGTSASLAISGLRSEDEADYYCAAWDDSLSGYVFGTGTKVTVLG (SEQ ID NO: 16)
NI-101.12F 6A	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRF SGSGSGTDFTLTISSLQPEDFATYYCQQSYSTPLTFGGGTKVEIKR (SEQ ID NO: 41)
NI-101.13A	DIQLTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSGSGS GTDFTLTISSLQPEDFATYYCQQSYSTRTFGQGTKVEIKR (SEQ ID NO: 44)
NI-101.13B	DIQLTQSPSTLSASVGDRVTITCRASQSISSWLAWYQQIPGKAPKLLIYKASSLESGVPSRFSGSGS GTEFTLTISSLQPDDFATYYCQQYNSYSRTFGQGTKLEIKR (SEQ ID NO: 45)

[0129] The corresponding nucleotide sequences encoding the above-identified variable regions are set forth in the attached sequence listing. An exemplary set of CDRs of the above amino acid sequences of the V_H and/or V_L region as depicted in Tables 2 and 3 are given in Table 4. However, as discussed in the following the person skilled in the art is well aware of the fact that in addition or alternatively CDRs may be used, which differ in their amino acid sequence from those set forth in Table 4 by one, two, three or even more amino acids in case of CDR2 and CDR3.

Table 4: Denomination of CDR protein sequences in Kabat Nomenclature of V_H and V_L regions of neoepitope specific antibodies.

Antibody	Variable heavy chain	Variable light chain
NI-101.10		
CDR1	SYGIH (SEQ ID NO: 17)	RASQSISSYLN (SEQ ID NO: 23)
CDR2	VIWFDGTKKYYTDSVKG (SEQ ID NO: 18)	AASSLQS (SEQ ID NO: 24)
CDR3	DRGIGARRGPYYMDV (SEQ ID NO: 19)	QQSYSTPLT (SEQ ID NO: 25)
NI-101.11		
CDR1	SYGMH (SEQ ID NO: 20)	RASQSISSYLN (SEQ ID NO: 23)
CDR2	VIWFDGTKKYYTDSVKG (SEQ ID NO: 21)	AASSLQS (SEQ ID NO: 24)
CDR3	DRGIGARRGPYYMDV (SEQ ID NO: 22)	QQSYSTPLT (SEQ ID NO: 25)
NI-101.12		
CDR1	SGSIC (SEQ ID NO: 26)	RASESINKYVN (SEQ ID NO: 29)
CDR2	WIGYFCYSGATFYTPSLRG (SEQ ID NO: 27)	AASSLQS (SEQ ID NO: 30)
CDR3	RAGENSGGIEPYYGMDV (SEQ ID NO: 28)	QQSYSAPYT (SEQ ID NO: 31)

NI-101.13		
CDR1	RRSYYWG (SEQ ID NO: 32)	SGSSSNIGSNYVY (SEQ ID NO: 35)
CDR2	SIHYSGSTYYNPSLKS (SEQ ID NO: 33)	RNNQRPS (SEQ ID NO: 36)
CDR3	SRWGSSWVFDY (SEQ ID NO: 34)	AAWDDSLSGYV (SEQ ID NO: 37)
NI-101.12F6A		
CDR1	SYGMH (SEQ ID NO: 20)	RASQSISSYLN (SEQ ID NO: 23)
CDR2	VIWFDGTKKYYTDSVKG (SEQ ID NO: 21)	AASSLQS (SEQ ID NO: 24)
CDR3	DRGIGARRGPYYMDV (SEQ ID NO: 22)	QQSYSTPLT (SEQ ID NO: 25)
NI-101.13A		
CDR1	RRSYYWG (SEQ ID NO: 32)	RASQSISSYLN (SEQ ID NO: 46)
CDR2	SIHYSGSTYYNPSLKS (SEQ ID NO: 33)	AASSLQS (SEQ ID NO: 47)
CDR3	SRWGSSWVFDY (SEQ ID NO: 34)	QQSYSTRT (SEQ ID NO: 48)
NI-101.13B		
CDR1	RRSYYWG (SEQ ID NO: 32)	RASQSISSWLA (SEQ ID NO: 49)
CDR2	SIHYSGSTYYNPSLKS (SEQ ID NO: 33)	KASSLES (SEQ ID NO: 50)
CDR3	SRWGSSWVFDY (SEQ ID NO: 34)	QQYNSYSRT (SEQ ID NO: 51)

[0130] In one embodiment, the antibody of the present invention is any one of the antibodies comprising an amino acid sequence of the V_H and/or V_L region as depicted in Tables 2 and 3. Alternatively, the antibody of the present invention is an antibody or antigen-binding fragment thereof, which competes for binding to the neoeptitope with at least one of the antibodies having the V_H and/or V_L region as depicted in Tables 2 and 3. Those antibodies may be murine as well, however, humanized, xenogeneic, or chimeric human-murine antibodies being preferred, in particular for therapeutic applications. An antigen-binding fragment of the antibody can be, for example, a single chain Fv fragment (scFv), a F(ab') fragment, a F(ab) fragment, and an $F(ab')_2$ fragment. For some applications only the variable regions of the antibodies are required, which can be obtained by treating the antibody with suitable reagents so as to generate Fab', Fab, or $F(ab'')_2$ portions. Such fragments are sufficient for use, for example, in immunodiagnostic procedures involving coupling the immunospecific portions of immunoglobulins to detecting reagents such as radioisotopes.

[0131] The present invention is further directed to isolated polypeptides which make up the antibodies of the present invention. Antibodies of the present invention comprise polypeptides, *e.g.*, amino acid sequences encoding specific antigen binding regions derived from immunoglobulin molecules. A polypeptide or amino acid sequence "derived from" a

designated protein refers to the origin of the polypeptide having a certain amino acid sequence. In certain cases, the polypeptide or amino acid sequence which is derived from a particular starting polypeptide or amino acid sequence has an amino acid sequence that is essentially identical to that of the starting sequence, or a portion thereof, wherein the portion consists of at least 10-20 amino acids, at least 20-30 amino acids, at least 30-50 amino acids, or which is otherwise identifiable to one of ordinary skill in the art as having its origin in the starting sequence.

[0132] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH), where at least one of VH-CDRs of the heavy chain variable region or at least two of the VH-CDRs of the heavy chain variable region are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2 or VH-CDR3 amino acid sequences from the antibodies disclosed herein. Alternatively, the VH-CDR1, VH-CDR2 and VH-CDR3 regions of the VH are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2 and VH-CDR3 amino acid sequences from the antibodies disclosed herein. Thus, according to this embodiment a heavy chain variable region of the invention has VH-CDR1, VH-CDR2 and VH-CDR3 polypeptide sequences related to the groups shown in Table 4, *supra*. While Table 4 shows VH-CDRs defined by the Kabat system, other CDR definitions, *e.g.*, VH-CDRs defined by the Chothia system, are also included in the present invention, and can be easily identified by a person of ordinary skill in the art using the data presented in Tables 2 and 3.

[0133] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2 and VH-CDR3 groups shown in Table 4.

[0134] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2 and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2 and VH-CDR3 groups shown in Table 4, except for one, two, three, four, five, or six amino acid substitutions in any one VH-CDR. In certain embodiments the amino acid substitutions are conservative.

[0135] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin light chain variable region (VL), where at least one of the VL-CDRs of the light chain variable region or at least two of the VL-CDRs of the light chain variable region are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2 or VL-CDR3 amino acid sequences from antibodies disclosed herein. Alternatively, the VL-CDR1, VL-CDR2 and VL-CDR3 regions of the VL are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2 and VL-CDR3 amino acid sequences from antibodies disclosed herein. Thus, according to this embodiment a light chain variable region of the invention has VL-CDR1, VL-CDR2 and VL-CDR3 polypeptide sequences related to the polypeptides shown in Table 4, *supra*. While Table 4 shows VL-CDRs defined by the Kabat system, other CDR definitions, *e.g.*, VL-CDRs defined by the Chothia system, are also included in the present invention.

[0136] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin light chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences which are identical to the VL-CDR1, VL-CDR2 and VL-CDR3 groups shown in Table 4.

[0137] In another embodiment, the present invention provides an isolated polypeptide comprising, consisting essentially of, or consisting of an immunoglobulin heavy chain variable region (VL) in which the VL-CDR1, VL-CDR2 and VL-CDR3 regions have polypeptide sequences which are identical to the VL-CDR1, VL-CDR2 and VL-CDR3 groups shown in Table 4, except for one, two, three, four, five, or six amino acid substitutions in any one VL-CDR. In certain embodiments the amino acid substitutions are conservative.

[0138] An immunoglobulin or its encoding cDNAs may be further modified. Thus, in a further embodiment the method of the present invention comprises any one of the step(s) of producing a chimeric antibody, humanized antibody, single-chain antibody, Fab-fragment, bi-specific antibody, fusion antibody, labeled antibody or an analog of any one of those. Corresponding methods are known to the person skilled in the art and are described, *e.g.*, in Harlow and Lane "Antibodies, A Laboratory Manual", CSH Press, Cold Spring Harbor, 1988. When derivatives of said antibodies are obtained by the phage display technique, surface plasmon resonance as employed in the BIAcore system can be used to increase the

efficiency of phage antibodies which bind to the same epitope as that of any one of the antibodies described herein (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13). The production of chimeric antibodies is described, for example, in international application WO89/09622. Methods for the production of humanized antibodies are described in, *e.g.*, European application EP-A1 0 239 400 and international application WO90/07861. A further source of antibodies to be utilized in accordance with the present invention are so-called xenogeneic antibodies. The general principle for the production of xenogeneic antibodies such as human antibodies in mice is described in, *e.g.*, international applications WO91/10741, WO94/02602, WO96/34096 and WO 96/33735. As discussed above, the antibody of the invention may exist in a variety of forms besides complete antibodies; including, for example, Fv, Fab and $F(ab)_2$, as well as in single chains; see *e.g.* international application WO88/09344.

[0139] The antibodies of the present invention or their corresponding immunoglobulin chain(s) can be further modified using conventional techniques known in the art, for example, by using amino acid deletion(s), insertion(s), substitution(s), addition(s), and/or recombination(s) and/or any other modification(s) known in the art either alone or in combination. Methods for introducing such modifications in the DNA sequence underlying the amino acid sequence of an immunoglobulin chain are well known to the person skilled in the art; see, *e.g.*, Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Modifications of the antibody of the invention include chemical and/or enzymatic derivatizations at one or more constituent amino acids, including side chain modifications, backbone modifications, and N- and C-terminal modifications including acetylation, hydroxylation, methylation, amidation, and the attachment of carbohydrate or lipid moieties, cofactors, and the like. Likewise, the present invention encompasses the production of chimeric proteins which comprise the described antibody or some fragment thereof at the amino terminus fused to heterologous molecule such as an immunostimulatory ligand at the carboxyl terminus; see, *e.g.*, international application WO00/30680 for corresponding technical details.

[0140] Additionally, the present invention encompasses small peptides including those containing a binding molecule as described above, for example containing the CDR3 region of the variable region of any one of the mentioned antibodies, in particular CDR3 of the heavy chain since it has frequently been observed that heavy chain CDR3 (HCDR3) is

the region having a greater degree of variability and a predominant participation in antigen-antibody interaction. Such peptides may easily be synthesized or produced by recombinant means to produce a binding agent useful according to the invention. Such methods are well known to those of ordinary skill in the art. Peptides can be synthesized for example, using automated peptide synthesizers which are commercially available. The peptides can be produced by recombinant techniques by incorporating the DNA expressing the peptide into an expression vector and transforming cells with the expression vector to produce the peptide.

[0141] Hence, the present invention relates to any binding molecule, *e.g.*, an antibody or binding fragment which is obtainable in accordance with above described means and display the mentioned properties, *i.e.* which specifically recognize a neoepitope. Such antibodies and binding molecules can be tested for their binding specificity and affinity by for example by using the method of isolating neoepitope specific binding molecules described hereinbefore.

[0142] As an alternative to obtaining immunoglobulins directly from the culture of immortalized B cells or B memory cells, the immortalized cells can be used as a source of rearranged heavy chain and light chain loci for subsequent expression and/or genetic manipulation. Rearranged antibody genes can be reverse transcribed from appropriate mRNAs to produce cDNA. If desired, the heavy chain constant region can be exchanged for that of a different isotype or eliminated altogether. The variable regions can be linked to encode single chain Fv regions. Multiple Fv regions can be linked to confer binding ability to more than one target or chimeric heavy and light chain combinations can be employed. Once the genetic material is available, design of analogs as described above which retain both their ability to bind the de-sired target is straightforward. Methods for the cloning of antibody variable regions and generation of recombinant antibodies are known to the person skilled in the art and are described, for example, Gilliland *et al.*, Tissue Antigens 47 (1996), 1-20; Doenecke *et al.*, Leukemia 11 (1997), 1787-1792.

[0143] Once the appropriate genetic material is obtained and, if desired, modified to encode an analog, the coding sequences, including those that encode, at a minimum, the variable regions of the heavy and light chain, can be inserted into expression systems contained on vectors which can be transfected into standard recombinant host cells. A variety of such host cells may be used; for efficient processing, however, mammalian cells

are preferred. Typical mammalian cell lines useful for this purpose include, but are not limited to, CHO cells, HEK 293 cells, or NSO cells.

[0144] The production of the antibody or analog is then undertaken by culturing the modified recombinant host under culture conditions appropriate for the growth of the host cells and the expression of the coding sequences. The antibodies are then recovered by isolating them from the culture. The expression systems are preferably designed to include signal peptides so that the resulting antibodies are secreted into the medium; however, intracellular production is also possible.

[0145] Once the target structure, *e.g.* the disease-associated protein has been tagged by the sample and respective binding molecule therein it may be identified by means and methods well known in the art, for example using mass spectrometric (MS) techniques such as those described in international application WO00/11208 and specifically those described in Hock *et al.*, Nat Med 8 (2002), 1270-1275; Hock *et al.*, Neuron 38 (2003), 547-554. Thus, in case the antibody identified in accordance with present invention produced *in vitro* binds to pathological structures, for example to beta-amyloid plaques in pathological brain area sections, but not significantly to healthy tissues, a promising antibody candidate has been identified whose molecular target structure can subsequently be enriched and purified via its binding properties to the antibody from pathological tissues and, as a result, can be identified and characterized by means of protein analytical and mass spectrometric methods, like for example MALDI/TOF (Williams, Methods Cell. Biol. 62 (2000), 449-453; Yates, J. Mass. Spectrom. 33 (1998), 1-19).

[0146] Accordingly, in another embodiment the present invention relates to an antigen which is recognized by the binding molecule, especially antibody of the present invention described hereinbefore, and which preferably is at least part of a disorder-associated protein.

[0147] In accordance with the above, the present invention also relates to a polynucleotide encoding the antigen or binding molecule of the present invention, in case of the antibody preferably at least a variable region of an immunoglobulin chain of the antibody described above. Typically, said variable region encoded by the polynucleotide comprises at least one complementarity determining region (CDR) of the V_H and/or V_L of the variable region of the said antibody. The person skilled in the art knows that each variable domain (the heavy chain V_H and light chain V_L) of an antibody comprises three hypervariable regions, sometimes called complementarity determining regions or "CDRs" flanked by four

relatively conserved framework regions or "FRs" and refer to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable regions or CDRs of the human IgG subtype of antibody comprise amino acid residues from residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the light chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy chain variable domain as described by Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed Public Health Service, National Institutes of Health, Bethesda, Md (1991) and/or those residues from a hypervariable loop, *e.g.* residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy chain variable domain as described by Chothia *et al.*, J. Mol. Biol. 196 (1987), 901-917. Framework or FR residues are those variable domain residues other than and bracketing the hypervaribale regions. The term "specific binding" refers to antibody binding to a predetermined antigen. Typically, the antibody binds with a dissociation constant (K_D) of 10^{-7} M or less, and binds to the predetermined antigen with a K_D that is at least twofold less than its K_D for binding to a nonspecific antigen (*e.g.*, BSA, casein, or any other specified polypeptide) other than the predetermined antigen. The phrases "an antibody recognizing an antigen" and "an antibody specific for an antigen" are used interchangeably herein with the term "an antibody which binds specifically to an antigen". As used herein "highly specific" binding means that the relative K_D of the antibody for the specific target epitope, *e.g.* neoepitope is at least 10-fold less than the K_D for binding that antibody to other ligands or to the native counterpart of the disease-associated protein.

[0148] The affinity or avidity of an antibody for an antigen can be determined experimentally using any suitable method; see, for example, Berzofsky *et al.*, "Antibody-Antigen Interactions" In Fundamental Immunology, Paul, W. E., Ed., Raven Press New York, N Y (1984), Kuby, Janis Immunology, W. H. Freeman and Company New York, N Y (1992), and methods described herein. General techniques for measuring the affinity of an antibody for an antigen include ELISA, RIA, and surface plasmon resonance. The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions, *e.g.*, salt concentration, pH. Thus, measurements of affinity and other antigen-binding parameters, *e.g.*, K_D, IC_{50}, are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

[0149] The person skilled in the art will readily appreciate that the variable domain of the antibody having the above-described variable domain can be used for the construction of other polypeptides or antibodies of desired specificity and biological function. Thus, the present invention also encompasses polypeptides and antibodies comprising at least one CDR of the above-described variable domain and which advantageously have substantially the same or similar binding properties as the antibody described in the appended examples. The person skilled in the art will readily appreciate that using the variable domains or CDRs described herein antibodies can be constructed according to methods known in the art, *e.g.*, as described in European patent applications EP 0 451 216 A1 and EP 0 549 581 A1. Furthermore, the person skilled in the art knows that binding affinity may be enhanced by making amino acid substitutions within the CDRs or within the hypervariable loops (Chothia and Lesk, J. Mol. Biol. 196 (1987), 901-917) which partially overlap with the CDRs as defined by Kabat. Thus, the present invention also relates to antibodies wherein one or more of the mentioned CDRs comprise one or more, preferably not more than two amino acid substitutions. Preferably, the antibody of the invention comprises in one or both of its immunoglobulin chains two or all three CDRs of the variable regions as set forth in Table 4.

[0150] Binding molecules, *e.g.*, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention, as known by those of ordinary skill in the art, can comprise a constant region which mediates one or more effector functions. For example, binding of the C1 component of complement to an antibody constant region may activate the complement system. Activation of complement is important in the opsonisation and lysis of cell pathogens. The activation of complement also stimulates the inflammatory response and may also be involved in autoimmune hypersensitivity. Further, antibodies bind to receptors on various cells via the Fc region, with a Fc receptor binding site on the antibody Fc region binding to a Fc receptor (FcR) on a cell. There are a number of Fc receptors which are specific for different classes of antibody, including IgG (gamma receptors), IgE (epsilon receptors), IgA (alpha receptors) and IgM (mu receptors). Binding of antibody to Fc receptors on cell surfaces triggers a number of important and diverse biological responses including engulfment and destruction of antibody-coated particles, clearance of immune complexes, lysis of antibody-coated target cells by killer cells (called antibody-dependent cell-mediated cytotoxicity, or ADCC), release of inflammatory mediators, placental transfer and control of immunoglobulin production.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6482

[0151] Accordingly, certain embodiments of the invention include an antibody, or antigen-binding fragment, variant, or derivative thereof, in which at least a fraction of one or more of the constant region domains has been deleted or otherwise altered so as to provide desired biochemical characteristics such as reduced effector functions, the ability to non-covalently dimerize, increased ability to localize at the site of a tumor, reduced serum half-life, or increased serum half-life when compared with a whole, unaltered antibody of approximately the same immunogenicity. For example, certain antibodies for use in the diagnostic and treatment methods described herein are domain deleted antibodies which comprise a polypeptide chain similar to an immunoglobulin heavy chain, but which lack at least a portion of one or more heavy chain domains. For instance, in certain antibodies, one entire domain of the constant region of the modified antibody will be deleted, for example, all or part of the CH2 domain will be deleted. In other embodiments, certain antibodies for use in the diagnostic and treatment methods described herein have a constant region, *e.g.*, an IgG heavy chain constant region, which is altered to eliminate glycosylation, referred to elsewhere herein as aglycosylated or "agly" antibodies. Such "agly" antibodies may be prepared enzymatically as well as by engineering the consensus glycosylation site(s) in the constant region. While not being bound by theory, it is believed that "agly" antibodies may have an improved safety and stability profile *in vivo*. Methods of producing aglycosylated antibodies, having desired effector function are found for example in WO 2005/018572, which is incorporated by reference in its entirety.

[0152] In certain antibodies, or antigen-binding fragments, variants, or derivatives thereof described herein, the Fc portion may be mutated to decrease effector function using techniques known in the art. For example, the deletion or inactivation (through point mutations or other means) of a constant region domain may reduce Fc receptor binding of the circulating modified antibody thereby increasing tumor localization. In other cases it may be that constant region modifications consistent with the instant invention moderate complement binding and thus reduce the serum half life and nonspecific association of a conjugated cytotoxin. Yet other modifications of the constant region may be used to modify disulfide linkages or oligosaccharide moieties that allow for enhanced localization due to increased antigen specificity or antibody flexibility. The resulting physiological profile, bioavailability and other biochemical effects of the modifications, such as tumor localization, biodistribution and serum half-life, may easily be measured and quantified using well know immunological techniques without undue experimentation.

[0153] Modified forms of antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can be made from whole precursor or parent antibodies using techniques known in the art. Exemplary techniques are discussed in more detail herein.

[0154] In certain embodiments both the variable and constant regions of the antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention are fully human. Fully human antibodies can be made using techniques that are known in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in US patents: 6,150,584; 6,458,592; 6,420,140. Other techniques are known in the art. Fully human antibodies can likewise be produced by various display technologies, *e.g.*, phage display or other viral display systems, as described in more detail elsewhere herein.

[0155] Antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can be made or manufactured using techniques that are known in the art. In certain embodiments, antibody molecules or fragments thereof are "recombinantly produced," *i.e.*, are produced using recombinant DNA technology. Exemplary techniques for making antibody molecules or fragments thereof are discussed in more detail elsewhere herein.

[0156] Antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention also include derivatives that are modified, *e.g.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from specifically binding to its cognate epitope. For example, but not by way of limitation, the antibody derivatives include antibodies that have been modified, *e.g.*, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the derivative may contain one or more non-classical amino acids.

[0157] In certain embodiments, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention will not elicit a deleterious immune response in the

animal to be treated, *e.g.*, in a human. In certain embodiments, binding molecules, *e.g.*, antibodies, or antigen-binding fragments thereof of the invention are derived from a patient, *e.g.*, a human patient, and are subsequently used in the same species from which they are derived, *e.g.*, human, alleviating or minimizing the occurrence of deleterious immune responses.

[0158] De-immunization can also be used to decrease the immunogenicity of an antibody. As used herein, the term "de-immunization" includes alteration of an antibody to modify T cell epitopes (*see, e.g.*, WO9852976A1, WO0034317A2). For example, VH and VL sequences from the starting antibody are analyzed and a human T cell epitope "map" from each V region showing the location of epitopes in relation to complementarity-determining regions (CDRs) and other key residues within the sequence. Individual T cell epitopes from the T cell epitope map are analyzed in order to identify alternative amino acid substitutions with a low risk of altering activity of the final antibody. A range of alternative VH and VL sequences are designed comprising combinations of amino acid substitutions and these sequences are subsequently incorporated into a range of binding polypeptides, *e.g.*, neo-epitope-specific antibodies or immunospecific fragments thereof for use in the diagnostic and treatment methods disclosed herein, which are then tested for function. Typically, between 12 and 24 variant antibodies are generated and tested. Complete heavy and light chain genes comprising modified V and human C regions are then cloned into expression vectors and the subsequent plasmids introduced into cell lines for the production of whole antibody. The antibodies are then compared in appropriate biochemical and biological assays, and the optimal variant is identified.

[0159] Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow *et al., Antibodies: A Laboratory* Manual, Cold Spring Harbor Laboratory Press, 2nd ed. (1988); Hammerling *et al.*, in: *Monoclonal Antibodies and T-Cell Hybridomas* Elsevier, N.Y., 563-681 (1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. Thus, the term "monoclonal antibody" is not limited to

antibodies produced through hybridoma technology. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art. In certain embodiments, antibodies of the present invention are derived from human B cells which have been immortalized via transformation with Epstein-Barr virus, as described herein. .

[0160] In the well known hybridoma process (Kohler *et al., Nature 256*:495 (1975)) the relatively short-lived, or mortal, lymphocytes from a mammal, *e.g.*, B cells derived from a human subject as described herein, are fused with an immortal tumor cell line (*e.g.*,. a myeloma cell line), thus, producing hybrid cells or "hybridomas" which are both immortal and capable of producing the genetically coded antibody of the B cell. The resulting hybrids are segregated into single genetic strains by selection, dilution, and regrowth with each individual strain comprising specific genes for the formation of a single antibody. They produce antibodies, which are homogeneous against a desired antigen and, in reference to their pure genetic parentage, are termed "monoclonal."

[0161] Hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. Those skilled in the art will appreciate that reagents, cell lines and media for the formation, selection and growth of hybridomas are commercially available from a number of sources and standardized protocols are well established. Generally, culture medium in which the hybridoma cells are growing is assayed for production of monoclonal antibodies against the desired antigen. The binding specificity of the monoclonal antibodies produced by hybridoma cells is determined by *in vitro* assays such as immunoprecipitation, radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA), or neoepitope binding assays as described herein. After hybridoma cells are identified that produce antibodies of the desired specificity, affinity and/or activity, the clones may be subcloned by limiting dilution procedures and grown by standard methods (Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press, pp 59-103 (1986)). It will further be appreciated that the monoclonal antibodies secreted by the subclones may be separated from culture medium, ascites fluid or serum by conventional purification procedures such as, for example, protein-A, hydroxylapatite chromatography, gel electrophoresis, dialysis or affinity chromatography.

[0162] Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and $F(ab')_2$ fragments may be produced recombinantly or by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to

produce Fab fragments) or pepsin (to produce $F(ab')_2$ fragments). $F(ab')_2$ fragments contain the variable region, the light chain constant region and the CH1 domain of the heavy chain.

[0163] Completely human antibodies, such as described herein, are particularly desirable for therapeutic treatment of human patients. Human antibodies can be made by a variety of methods known in the art including phage display methods described above using antibody libraries derived from human immunoglobulin sequences. *See also*, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety. Human antibodies of the present invention are isolated, *e.g.*, from patients who are symptom free but affected with the risk of developing a disorder, *e.g.*, Alzheimer's disease, or a patient with the disorder but with an unusually stable disease course.

[0164] In another embodiment, DNA encoding desired monoclonal antibodies may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The isolated and subcloned hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into prokaryotic or eukaryotic host cells such as, but not limited to, *E. coli* cells, simian COS cells, Chinese Hamster Ovary (CHO) cells or myeloma cells that do not otherwise produce immunoglobulins. More particularly, the isolated DNA (which may be synthetic as described herein) may be used to clone constant and variable region sequences for the manufacture antibodies as described in Newman *et al.*, U.S. Pat. No. 5,658,570, filed January 25, 1995, which is incorporated by reference herein. Essentially, this entails extraction of RNA from the selected cells, conversion to cDNA, and amplification by PCR using Ig specific primers. Suitable primers for this purpose are also described in U.S. Pat. No. 5,658,570. As will be discussed in more detail below, transformed cells expressing the desired antibody may be grown up in relatively large quantities to provide clinical and commercial supplies of the immunoglobulin.

[0165] In one embodiment, an antibody of the invention comprises at least one heavy or light chain CDR of an antibody molecule. In another embodiment, an antibody of the invention comprises at least two CDRs from one or more antibody molecules. In another embodiment, an antibody of the invention comprises at least three CDRs from one or more antibody molecules. In another embodiment, an antibody of the invention comprises at

least four CDRs from one or more antibody molecules. In another embodiment, an antibody of the invention comprises at least five CDRs from one or more antibody molecules. In another embodiment, an antibody of the invention comprises at least six CDRs from one or more antibody molecules. Exemplary antibody molecules comprising at least one CDR that can be included in the subject antibodies are described herein.

[0166] In a specific embodiment, the amino acid sequence of the heavy and/or light chain variable domains may be inspected to identify the sequences of the complementarity determining regions (CDRs) by methods that are well know in the art, *e.g.*, by comparison to known amino acid sequences of other heavy and light chain variable regions to determine the regions of sequence hypervariability. Using routine recombinant DNA techniques, one or more of the CDRs may be inserted within framework regions, *e.g.*, into human framework regions. The framework regions may be naturally occurring or consensus framework regions, and preferably human framework regions (*see, e.g.*, Chothia *et al.*, *J. Mol. Biol. 278*:457-479 (1998) for a listing of human framework regions). In certain embodiments, the polynucleotide generated by the combination of the framework regions and CDRs encodes an antibody that specifically binds to at least one epitope of a desired polypeptide. In certain embodiments, one or more amino acid substitutions may be made within the framework regions, to, *e.g.*, improve binding of the antibody to its antigen. Additionally, such methods may be used to make amino acid substitutions or deletions of one or more variable region cysteine residues participating in an intrachain disulfide bond to generate antibody molecules lacking one or more intrachain disulfide bonds. Other alterations to the polynucleotide are encompassed by the present invention and within the skill of the art.

[0167] Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,694,778; Bird, *Science 242*:423-442 (1988); Huston *et al.*, *Proc. Natl. Acad. Sci. USA 85*:5879-5883 (1988); and Ward *et al.*, *Nature 334*:544-554 (1989)) can be adapted to produce single chain antibodies. Single chain antibodies are formed by linking the heavy and light chain fragments of the Fv region via an amino acid bridge, resulting in a single chain antibody. Techniques for the assembly of functional Fv fragments in *E coli* may also be used (Skerra *et al.*, *Science 242*:1038-1041 (1988)).

[0168] In another embodiment, lymphocytes can be selected by micromanipulation and the variable genes isolated. For example, peripheral blood mononuclear cells can be isolated from an immunized or naturally immune mammal, *e.g.*, a human, and cultured for about 7

days *in vitro*. The cultures can be screened for specific IgGs that meet the screening criteria. Cells from positive wells can be isolated. Individual Ig-producing B cells can be isolated by FACS or by identifying them in a complement-mediated hemolytic plaque assay. Ig-producing B cells can be micromanipulated into a tube and the VH and VL genes can be amplified using, *e.g.*, RT-PCR. The VH and VL genes can be cloned into an antibody expression vector and transfected into cells (*e.g.*, eukaryotic or prokaryotic cells) for expression.

[0169] Alternatively, antibody-producing cell lines may be selected and cultured using techniques well known to the skilled artisan. Such techniques are described in a variety of laboratory manuals and primary publications. In this respect, techniques suitable for use in the invention as described below are described in *Current Protocols in Immunology*, Coligan *et al.*, Eds., Green Publishing Associates and Wiley-Interscience, John Wiley and Sons, New York (1991) which is herein incorporated by reference in its entirety, including supplements.

[0170] Antibodies of the present invention can be produced by any method known in the art for the synthesis of antibodies, in particular, by chemical synthesis or preferably, by recombinant expression techniques as described herein.

[0171] In one embodiment, an antibody, or antigen-binding fragment, variant, or derivative thereof of the invention comprises a synthetic constant region wherein one or more domains are partially or entirely deleted ("domain-deleted antibodies"). In certain embodiments compatible modified antibodies will comprise domain deleted constructs or variants wherein the entire CH2 domain has been removed (ΔCH2 constructs). For other embodiments a short connecting peptide may be substituted for the deleted domain to provide flexibility and freedom of movement for the variable region. Those skilled in the art will appreciate that such constructs are particularly preferred due to the regulatory properties of the CH2 domain on the catabolic rate of the antibody. Domain deleted constructs can be derived using a vector encoding an IgG_1 human constant domain (*see, e.g.*, WO 02/060955A2 and WO02/096948A2). This vector is engineered to delete the CH2 domain and provide a synthetic vector expressing a domain deleted IgG_1 constant region.

[0172] In certain embodiments, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the present invention are minibodies. Minibodies can be made using methods described in the art (*see, e.g.*, US patent 5,837,821 or WO 94/09817A1).

[0173] In one embodiment, an antibody, or antigen-binding fragment, variant, or derivative thereof of the invention comprises an immunoglobulin heavy chain having deletion or substitution of a few or even a single amino acid as long as it permits association between the monomeric subunits. For example, the mutation of a single amino acid in selected areas of the CH2 domain may be enough to substantially reduce Fc binding and thereby increase tumor localization. Similarly, it may be desirable to simply delete that part of one or more constant region domains that control the effector function (*e.g.* complement binding) to be modulated. Such partial deletions of the constant regions may improve selected characteristics of the antibody (serum half-life) while leaving other desirable functions associated with the subject constant region domain intact. Moreover, as alluded to above, the constant regions of the disclosed antibodies may be synthetic through the mutation or substitution of one or more amino acids that enhances the profile of the resulting construct. In this respect it may be possible to disrupt the activity provided by a conserved binding site (*e.g.* Fc binding) while substantially maintaining the configuration and immunogenic profile of the modified antibody. Yet other embodiments comprise the addition of one or more amino acids to the constant region to enhance desirable characteristics such as effector function or provide for more cytotoxin or carbohydrate attachment. In such embodiments it may be desirable to insert or replicate specific sequences derived from selected constant region domains.

[0174] The present invention also provides antibodies that comprise, consist essentially of, or consist of, variants (including derivatives) of antibody molecules (*e.g.*, the VH regions and/or VL regions) described herein, which antibodies or fragments thereof immunospecifically bind to a disorder-associated polypeptide or fragment or variant thereof. Standard techniques known to those of skill in the art can be used to introduce mutations in the nucleotide sequence encoding an antibody, including, but not limited to, site-directed mutagenesis and PCR-mediated mutagenesis which result in amino acid substitutions. Preferably, the variants (including derivatives) encode less than 50 amino acid substitutions, less than 40 amino acid substitutions, less than 30 amino acid substitutions, less than 25 amino acid substitutions, less than 20 amino acid substitutions, less than 15 amino acid substitutions, less than 10 amino acid substitutions, less than 5

amino acid substitutions, less than 4 amino acid substitutions, less than 3 amino acid substitutions, or less than 2 amino acid substitutions relative to the reference VH region, VH-CDR1, VH-CDR2, VH-CDR3, VL region, VL-CDR1, VL-CDR2, or VL-CDR3. A "conservative amino acid substitution" is one in which the amino acid residue is replaced with an amino acid residue having a side chain with a similar charge. Families of amino acid residues having side chains with similar charges have been defined in the art. These families include amino acids with basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Alternatively, mutations can be introduced randomly along all or part of the coding sequence, such as by saturation mutagenesis, and the resultant mutants can be screened for biological activity to identify mutants that retain activity (*e.g.*, the ability to bind an disorder-associated polypeptide).

[0175] For example, it is possible to introduce mutations only in framework regions or only in CDR regions of an antibody molecule. Introduced mutations may be silent or neutral missense mutations, *e.g.*, have no, or little, effect on an antibody's ability to bind antigen, indeed some such mutations do not alter the amino acid sequence whatsoever. These types of mutations may be useful to optimize codon usage, or improve a hybridoma's antibody production. Codon-optimized coding regions encoding antibodies of the present invention are disclosed elsewhere herein. Alternatively, non-neutral missense mutations may alter an antibody's ability to bind antigen. The location of most silent and neutral missense mutations is likely to be in the framework regions, while the location of most non-neutral missense mutations is likely to be in CDR, though this is not an absolute requirement. One of skill in the art would be able to design and test mutant molecules with desired properties such as no alteration in antigen binding activity or alteration in binding activity (*e.g.*, improvements in antigen binding activity or change in antibody specificity). Following mutagenesis, the encoded protein may routinely be expressed and the functional and/or biological activity of the encoded protein, (*e.g.*, ability to immunospecifically bind at least one epitope of a disorder-associated polypeptide) can be determined using techniques described herein or by routinely modifying techniques known in the art.

IV. Polynucleotides Encoding Antibodies

[0176] In accordance with the above, the present invention also relates to a polynucleotide encoding a binding molecule of the present invention,, *e.g.*, an antibody. In case of the antibodythe polynucleotide may encode at least a variable region of an immunoglobulin chain of the antibody described above. The polynucleotide of the invention encoding the above described antibody may be, *e.g.*, DNA, cDNA, RNA or synthetically produced DNA or RNA or a recombinantly produced chimeric nucleic acid molecule comprising any of those polynucleotides either alone or in combination. Preferably said polynucleotide is part of a vector. Such vectors may comprise further genes such as marker genes which allow for the selection of said vector in a suitable host cell and under suitable conditions. Preferably, the polynucleotide of the invention is operatively linked to expression control sequences allowing expression in prokaryotic or eukaryotic cells. Expression of said polynucleotide comprises transcription of the polynucleotide into a translatable mRNA. Regulatory elements ensuring expression in eukaryotic cells, preferably mammalian cells, are well known to those skilled in the art. They usually comprise regulatory sequences ensuring initiation of transcription and optionally poly-A signals ensuring termination of transcription and stabilization of the transcript. Additional regulatory elements may include transcriptional as well as translational enhancers, and/or naturally associated or heterologous promoter regions.

[0177] A polynucleotide encoding an antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of any polyribonucleotide or polydeoxribonucleotide, which may be unmodified RNA or DNA or modified RNA or DNA. For example, a polynucleotide encoding an antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, and RNA that is mixture of single- and double-stranded regions, hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, a polynucleotide encoding an antibody, or antigen-binding fragment, variant, or derivative thereof can be composed of triple-stranded regions comprising RNA or DNA or both RNA and DNA. A polynucleotide encoding an antibody, or antigen-binding fragment, variant, or derivative thereof may also contain one or more modified bases or DNA or RNA backbones modified

for stability or for other reasons. "Modified" bases include, for example, tritylated bases and unusual bases such as inosine. A variety of modifications can be made to DNA and RNA; thus, "polynucleotide" embraces chemically, enzymatically, or metabolically modified forms.

[0178] An isolated polynucleotide encoding a non-natural variant of a polypeptide derived from an immunoglobulin (*e.g.*, an immunoglobulin heavy chain portion or light chain portion) can be created by introducing one or more nucleotide substitutions, additions or deletions into the nucleotide sequence of the immunoglobulin such that one or more amino acid substitutions, additions or deletions are introduced into the encoded protein. Mutations may be introduced by standard techniques, such as site-directed mutagenesis and PCR-mediated mutagenesis. Preferably, conservative amino acid substitutions are made at one or more non-essential amino acid residues.

[0179] As is well known, RNA may be isolated from the original hybridoma cells or from other transformed cells by standard techniques, such as guanidinium isothiocyanate extraction and precipitation followed by centrifugation or chromatography. Where desirable, mRNA may be isolated from total RNA by standard techniques such as chromatography on oligo dT cellulose. Suitable techniques are familiar in the art.

[0180] In one embodiment, cDNAs that encode the light and the heavy chains of the antibody may be made, either simultaneously or separately, using reverse transcriptase and DNA polymerase in accordance with well known methods. PCR may be initiated by consensus constant region primers or by more specific primers based on the published heavy and light chain DNA and amino acid sequences. As discussed above, PCR also may be used to isolate DNA clones encoding the antibody light and heavy chains. In this case the libraries may be screened by consensus primers or larger homologous probes, such as mouse constant region probes.

[0181] DNA, typically plasmid DNA, may be isolated from the cells using techniques known in the art, restriction mapped and sequenced in accordance with standard, well known techniques set forth in detail, *e.g.*, in the foregoing references relating to recombinant DNA techniques. Of course, the DNA may be synthetic according to the present invention at any point during the isolation process or subsequent analysis.

[0182] In one embodiment, the present invention provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH), where at least one of the CDRs of the

heavy chain variable region or at least two of the VH-CDRs of the heavy chain variable region are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2, or VH-CDR3 amino acid sequences from the antibodies disclosed herein. Alternatively, the VH-CDR1, VH-CDR2, and VH-CDR3 regions of the VH are at least 80%, 85%, 90% or 95% identical to reference heavy chain VH-CDR1, VH-CDR2, and VH-CDR3 amino acid sequences from the antibodies disclosed herein. Thus, according to this embodiment a heavy chain variable region of the invention has VH-CDR1, VH-CDR2, or VH-CDR3 polypeptide sequences related to the polypeptide sequences shown in Table 4.

[0183] In another embodiment, the present invention provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin light chain variable region (VL), where at least one of the VL-CDRs of the light chain variable region or at least two of the VL-CDRs of the light chain variable region are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2, or VL-CDR3 amino acid sequences from the antibodies disclosed herein. Alternatively, the VL-CDR1, VL-CDR2, and VL-CDR3 regions of the VL are at least 80%, 85%, 90% or 95% identical to reference light chain VL-CDR1, VL-CDR2, and VL-CDR3 amino acid sequences from the antibodies disclosed herein. Thus, according to this embodiment a light chain variable region of the invention has VL-CDR1, VL-CDR2, or VL-CDR3 polypeptide sequences related to the polypeptide sequences shown in Table 4.

[0184] In another embodiment, the present invention provides an isolated polynucleotide comprising, consisting essentially of, or consisting of a nucleic acid encoding an immunoglobulin heavy chain variable region (VH) in which the VH-CDR1, VH-CDR2, and VH-CDR3 regions have polypeptide sequences which are identical to the VH-CDR1, VH-CDR2, and VH-CDR3 groups shown in Table 4.

[0185] As known in the art, "sequence identity" between two polypeptides or two polynucleotides is determined by comparing the amino acid or nucleic acid sequence of one polypeptide or polynucleotide to the sequence of a second polypeptide or polynucleotide. When discussed herein, whether any particular polypeptide is at least about 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% identical to another polypeptide can be determined using methods and computer programs/software known in the art such as, but not limited to, the BESTFIT program (Wisconsin Sequence Analysis Package,

Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive, Madison, WI 53711). BESTFIT uses the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2:482-489 (1981), to find the best segment of homology between two sequences. When using BESTFIT or any other sequence alignment program to determine whether a particular sequence is, for example, 95% identical to a reference sequence according to the present invention, the parameters are set, of course, such that the percentage of identity is calculated over the full length of the reference polypeptide sequence and that gaps in homology of up to 5% of the total number of amino acids in the reference sequence are allowed.

Table 5: Polynucleotide sequences of the V_H region of neoepitope specific antibodies.

Antibody	Variable heavy chain sequence
NI-101.10 (SEQ ID NO:3)	GAGGTGCAGCTAGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCC TGTGCAGCGTCTGGATTCGCCTTCAGTAGCTATGGCATACACTGGGTCCGCCAGGCTCCAGGCA AGGGGCTGGAGTGGGTGGCAGTTATATGGTTTGATGGAACTAAAAAATACTATACAGACTCCGTG AAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACACTGTATCTGCAAATGAACACCCT GAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATAGGGGTATAGGAGCTCGGCGGGG GCCGTACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCA
NI-101.11 (SEQ ID NO:56)	GAGGTGCAGCTGGTGCAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCC TGTGCAGCGTCTGGATTCGCCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCA AGGGGCTGGAGTGGGTGGCAGTTATATGGTTTGATGGAACTAAAAAATACTATACAGACTCCGTG AAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACACTGTATCTGCAAATGAACACCCT GAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATAGGGGTATAGGAGCTCGGCGGGG GCCGTACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCA
NI-101.11 (SEQ ID NO:5) (codon-optimized)	GAGGTGCAGCTGGTGCAGAGCGGCGGCGGCGTGGTGCAGCCCGGCCGGAGCCTGCGGCTGAG CTGCGCCGCCAGCGGCTTCGCCTTCAGCAGCTACGGCATGCACTGGGTGCGGCAGGCCCCCGG CAAGGGCCTGGAGTGGGTGGCCGTGATCTGGTTCGACGGCACCAAGAAGTACTACACCGACAGC GTGAAGGGCCGGTTCACCATCAGCCGGGACAACAGCAAGAACACCCTGTACCTGCAGATGAACA CCCTGCGGGCCGAGGACACCGCCGTGTACTACTGCGCCCGGGACCGGGGCATCGGCGCCCGG CGGGGCCCCTACTACATGGACGTGTGGGGCAAGGGCACCACCGTGACCGTGAGCAGC
NI-101.12 (SEQ ID NO:9)	GAGGTGCAGCTGGTGGAGAGCGGCCCCGGCCTGGTGAAGCCCGCCGAGACCCTGAGCCTGACC TGCACCGTGAGCGGCGGCAGCATCCGGAGCGGCAGCATCTGCTGGTACTGGATCCGGCAGCCC CCCGGCAAGGGCCTGGAGTGGATCGGCTACTTCTGCTACAGCGGCGCCACCTTCTACACCCCCA GCCTGCGGGGCCGGCTGACCATCAGCGTGGACGCCAGCAAGAACCAGCTGAGCCTGAGCCTGA GCAGCGTGACCGCCGCCGACACCGCCGTGTACTACTGCGCCCGGCGGGCCGGCGAGAACAGC GGCGGCATCGAGCCCTACTACGGCATGGACGTGTGGGGCCAGGGCACCACCGTGACCGTGAGC AGC

NI-101.13 (SEQ ID NO:13)	CAGGTACAGCTGCAGGAGTCAGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCT GCACTGTCTCTGGTGGCTCCATCAGCAGAAGAAGTTACTACTGGGGCTGGATCCGCCAGTCCCC AGGGAAGGGGCTGGAGTGGAGTGGAAGTATCCATTATAGCGGGAGCACCTACTACAACCCGTCC CTCAAGAGTCGAGTCACCATATCTGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGAGCTC TGTTACCGCCGCAGACACGGCTGTCTATTACTGTGCGAGATCACGTTGGGGCAGCAGCTGGGTA TTTGACTACTGGGGCCAGGGCACACTGGTCACCGTCTCTTCG
NI-101.12F 6A (SEQ ID NO:38)	CAGGTGCAGCTGGTGGAGTCTGGGGGAGGCGTGGTCCAGCCTGGGAGGTCCCTGAGACTCTCC TGTGCAGCGTCTGGATTCGCCTTCAGTAGCTATGGCATGCACTGGGTCCGCCAGGCTCCAGGCA AGGGGCTGGAGTGGGTGGCAGTTATATGGTTTGATGGAACTAAAAAATACTATACAGACTCCGTG AAGGGCAGATTCACCATCTCCAGAGACAATTCCAAGAACACACTGTATCTGCAAATGAACACCCT GAGAGCCGAGGACACGGCTGTGTATTACTGTGCGAGAGATAGGGGTATAGGAGCTCGGCGGGG GCCGTACTACATGGACGTCTGGGGCAAAGGGACCACGGTCACCGTCTCCTCA
NI-101.13A (SEQ ID NO:52)	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCT GCACTGTCTCTGGTGGCTCCATCAGCAGAAGAAGTTACTACTGGGGCTGGATCCGCCAGTCCCC AGGGAAGGGGCTGGAGTGGAGTGGAAGTATCCATTATAGCGGGAGCACCTACTACAACCCGTCC CTCAAGAGTCGAGTCACCATATCTGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGAGCTC TGTTACCGCCGCAGACACGGCTGTCTATTACTGTGCGAGATCACGTTGGGGCAGCAGCTGGGTA TTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCG
NI-101.13B (SEQ ID NO:53)	CAGGTGCAGCTGCAGGAGTCGGGCCCAGGACTGGTGAAGCCTTCGGAGACCCTGTCCCTCACCT GCACTGTCTCTGGTGGCTCCATCAGCAGAAGAAGTTACTACTGGGGCTGGATCCGCCAGTCCCC AGGGAAGGGGCTGGAGTGGAGTGGAAGTATCCATTATAGCGGGAGCACCTACTACAACCCGTCC CTCAAGAGTCGAGTCACCATATCTGTAGACACGTCCAAGAACCAGTTCTCCCTGAAACTGAGCTC TGTTACCGCCGCAGACACGGCTGTCTATTACTGTGCGAGATCACGTTGGGGCAGCAGCTGGGTA TTTGACTACTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCG

Table 6: Polynucleotide sequences of the V_L region of neoepitope specific antibodies.

Antibody	Variable light chain sequence (kappa or lambda)
NI-101.10 and NI-101.11 (SEQ ID NO:7)	GAAATTGTGCTGACTCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCACT TGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAACAGAAACCAGGGAAAGCCCC TAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGTG GATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTATTACT GTCAGCAGAGTTACAGTACCCCTCTCACTTTCGGCGGAGGGACCAAGCTCGAGATCAAACGTAC G
NI-101.12 (SEQ ID NO:11)	GACGAGATCGTGCTGACCCAGAGCCCCAGCAGCCTGAGCGCCAGCATCGGCGACCGGGTGACC ATCACCTGCCGGGCCAGCGAGAGCATCAACAAGTACGTGAACTGGTACCAGCAGAAGCCCGGCA AGGCCCCCAAGCTGCTGATCTACGCCGCCAGCAGCCTGCAGAGCGGCGCCCCCAGCCGGGTGA GCGGCAGCGGCTTCGGCCGGGACTTCAGCCTGACCATCAGCGGCCTGCAGGCCGAGGACTTCG GCGCCTACTTCTGCCAGCAGAGCTACAGCGCCCCCTACACCTTCGGCCAGGGCACCAAGGTGGA GATCAAGCGGACC
NI-101.13 (SEQ ID	CAGAGCGTGCTGACCCAGCCGCCGAGCGCGAGCGGCACCCCGGGCCAGCGCGTGACCATTAGC TGCAGCGGCAGCAGCAGCAACATTGGCAGCAACTATGTGTATTGGTATCAGCAGCCGCCGGGCA

NO:15)	CCGCGCCGAAACTGCTGATTTATCGCAACAACCAGCGCCCGAGCGGCGTGCCGGATCGCTTTAG CGGCAGCAAAAGCGGCACCAGCGCGAGCCTGGCGATTAGCGGCCTGCGCAGCGAAGATGAAGC GGATTATTATTGCGCGGCGTGGGATGATAGCCTGAGCGGCTATGTGTTTGGCACCGGCACCAAA GTGACCGTGCTG
NI-101.12F 6A (SEQ ID NO:40)	GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAC TTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAACAGAAACCAGGGAAAGCCC CTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGT GGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTATTAC TGTCAGCAGAGTTACAGTACCCCTCTCACTTTCGGCGGAGGGACCAAGGTGGAGATCAAACGT
NI-101.13A (SEQ ID NO:54)	GACATCCAGTTGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAC TTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGAAAGCCC CTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCAAGGTTCAGTGGCAGT GGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTAC TGTCAACAGAGTTACAGTACCAGAACGTTCGGCCAAGGGACCAAGGTGGAGATCAAACGTACG
NI-101.13B (SEQ ID NO:55)	GACATCCAGTTGACCCAGTCTCCTTCCACCCTGTCTGCATCTGTAGGAGACAGAGTCACCATCAC TTGCCGGGCCAGTCAGAGTATTAGTAGCTGGTTGGCCTGGTATCAGCAGATTCCAGGGAAAGCC CCTAAGCTCCTGATCTATAAGGCGTCTAGTTTAGAAAGTGGGGTCCCATCAAGGTTCAGCGGCAG TGGATCTGGGACAGAATTCACTCTCACCATCAGCAGCCTGCAGCCTGATGATTTTGCAACTTATTA CTGCCAACAGTATAATAGTTATTCTCGAACGTTCGGCCAAGGGACCAAGCTGGAGATCAAACGTA CG

[0186] In this respect, the person skilled in the art will readily appreciate that the polynucleotides encoding at least the variable domain of the light and/or heavy chain may encode the variable domains of both immunoglobulin chains or only one. Likewise, said polynucleotides may be under the control of the same promoter or may be separately controlled for expression. Possible regulatory elements permitting expression in prokaryotic host cells comprise, *e.g.*, the P_L, lac, trp or tac promoter in E. coli, and examples for regulatory elements permitting expression in eukaryotic host cells are the AOX1 or GAL1 promoter in yeast or the CMV-, SV40- , RSV-promoter, CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells. Beside elements which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide. Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the polynucleotide of the invention and are well known in the art. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination sequences, and preferably, a

leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including a C- or N-terminal identification peptide imparting desired characteristics, *e.g.*, stabilization or simplified purification of expressed recombinant product. In this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (Invitrogen), or pSPORT1 (GIBCO BRL). Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and, as desired, the collection and purification of the immunoglobulin light chains, heavy chains, light/heavy chain dimers or intact antibodies, binding fragments or other immunoglobulin forms may follow; see, Beychok, Cells of Immunoglobulin Synthesis, Academic Press, N.Y., (1979).

[0187] The present invention also includes fragments of the polynucleotides of the invention, as described elsewhere. Additionally polynucleotides which encode fusion polynucleotides, Fab fragments, and other derivatives, as described herein, are also contemplated by the invention.

[0188] The polynucleotides may be produced or manufactured by any method known in the art. For example, if the nucleotide sequence of the antibody is known, a polynucleotide encoding the antibody may be assembled from chemically synthesized oligonucleotides (*e.g.*, as described in Kutmeier *et al.*, *BioTechniques 17*:242 (1994)), which, briefly, involves the synthesis of overlapping oligonucleotides containing portions of the sequence encoding the antibody, annealing and ligating of those oligonucleotides, and then amplification of the ligated oligonucleotides by PCR.

[0189] Alternatively, a polynucleotide encoding an antibody, or antigen-binding fragment, variant, or derivative thereof may be generated from nucleic acid from a suitable source. If a clone containing a nucleic acid encoding a particular antibody is not available, but the sequence of the antibody molecule is known, a nucleic acid encoding the antibody may be chemically synthesized or obtained from a suitable source (*e.g.*, an antibody cDNA library, or a cDNA library generated from, or nucleic acid, preferably poly A+RNA, isolated from, any tissue or cells expressing the neoantigen-specific antibody, such as hybridoma cells

selected to express an antibody) by PCR amplification using synthetic primers hybridizable to the 3' and 5' ends of the sequence or by cloning using an oligonucleotide probe specific for the particular gene sequence to identify, *e.g.*, a cDNA clone from a cDNA library that encodes the antibody. Amplified nucleic acids generated by PCR may then be cloned into replicable cloning vectors using any method well known in the art.

[0190] Once the nucleotide sequence and corresponding amino acid sequence of the antibody, or antigen-binding fragment, variant, or derivative thereof is determined, its nucleotide sequence may be manipulated using methods well known in the art for the manipulation of nucleotide sequences, *e.g.*, recombinant DNA techniques, site directed mutagenesis, PCR, etc. (see, for example, the techniques described in Sambrook *et al.*, *Molecular Cloning, A Laboratory Manual*, 2d Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1990) and Ausubel *et al.*, eds., *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1998), which are both incorporated by reference herein in their entireties), to generate antibodies having a different amino acid sequence, for example to create amino acid substitutions, deletions, and/or insertions.

V. Expression of Antibody Polypeptides

[0191] The present invention also involves a method for producing cells capable of expressing an antibody of the invention or its corresponding immunoglobulin chain(s) comprising genetically engineering cells with the polynucleotide or with the vector of the invention. The cells obtainable by the method of the invention can be used, for example, to test the interaction of the antibody of the invention with its antigen.

[0192] Following manipulation of the isolated genetic material to provide antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention, the polynucleotides encoding the antibodies are typically inserted in an expression vector for introduction into host cells that may be used to produce the desired quantity of antibody.

[0193] Recombinant expression of an antibody, or fragment, derivative or analog thereof, *e.g.*, a heavy or light chain of an antibody which binds to a target molecule described herein. Once a polynucleotide encoding an antibody molecule or a heavy or light chain of an antibody, or portion thereof (preferably containing the heavy or light chain variable domain), of the invention has been obtained, the vector for the production of the antibody molecule may be produced by recombinant DNA technology using techniques well known in the art. Thus, methods for preparing a protein by expressing a polynucleotide containing

an antibody encoding nucleotide sequence are described herein. Methods which are well known to those skilled in the art can be used to construct expression vectors containing antibody coding sequences and appropriate transcriptional and translational control signals. These methods include, for example, *in vitro* recombinant DNA techniques, synthetic techniques, and *in vivo* genetic recombination. The invention, thus, provides replicable vectors comprising a nucleotide sequence encoding an antibody molecule of the invention, or a heavy or light chain thereof, or a heavy or light chain variable domain, operably linked to a promoter. Such vectors may include the nucleotide sequence encoding the constant region of the antibody molecule (*see, e.g.*, PCT Publication WO 86/05807; PCT Publication WO 89/01036; and U.S. Pat. No. 5,122,464) and the variable domain of the antibody may be cloned into such a vector for expression of the entire heavy or light chain.

[0194] The present invention relates to vectors, particularly plasmids, cosmids, viruses and bacteriophages used conventionally in genetic engineering that comprise a polynucleotide encoding the antigen or preferably a variable domain of an immunoglobulin chain of an antibody of the invention; optionally in combination with a polynucleotide of the invention that encodes the variable domain of the other immunoglobulin chain of the antibody of the in-vention. Preferably, said vector is an expression vector and/or a gene transfer or targeting vector. Expression vectors derived from viruses such as retroviruses, vaccinia virus, adeno-associated virus, herpes viruses, or bovine papilloma virus, may be used for delivery of the polynucleotides or vector of the invention into targeted cell population. Methods which are well known to those skilled in the art can be used to construct recombinant viral vectors; see, for example, the techniques described in Sambrook, Molecular Cloning A Laboratory Manual, Cold Spring Harbor Laboratory (1989) N.Y. and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. The vectors containing the polynucleotides of the invention (*e.g.*, the heavy and/or light variable domain(s) of the immunoglobulin chains encoding sequences and expression control sequences) can be transferred into the host cell by well known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts; see Sambrook, *supra.*

[0195] The term "vector" or "expression vector" is used herein to mean vectors used in accordance with the present invention as a vehicle for introducing into and expressing a desired gene in a host cell. As known to those skilled in the art, such vectors may easily be selected from the group consisting of plasmids, phages, viruses and retroviruses. In general, vectors compatible with the instant invention will comprise a selection marker, appropriate restriction sites to facilitate cloning of the desired gene and the ability to enter and/or replicate in eukaryotic or prokaryotic cells.

[0196] For the purposes of this invention, numerous expression vector systems may be employed. For example, one class of vector utilizes DNA elements which are derived from animal viruses such as bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (RSV, MMTV or MOMLV) or SV40 virus. Others involve the use of polycistronic systems with internal ribosome binding sites. Additionally, cells which have integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow selection of transfected host cells. The marker may provide for prototrophy to an auxotrophic host, biocide resistance (*e.g.*, antibiotics) or resistance to heavy metals such as copper. The selectable marker gene can either be directly linked to the DNA sequences to be expressed, or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include signal sequences, splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0197] In particularly preferred embodiments the cloned variable region genes are inserted into an expression vector along with the heavy and light chain constant region genes (preferably human) synthetic as discussed above. In one embodiment, this is effected using a proprietary expression vector of Biogen IDEC, Inc., referred to as NEOSPLA (disclosed in U.S. patent 6,159,730). This vector contains the cytomegalovirus promoter/enhancer, the mouse beta globin major promoter, the SV40 origin of replication, the bovine growth hormone polyadenylation sequence, neomycin phosphotransferase exon 1 and exon 2, the dihydrofolate reductase gene and leader sequence. This vector has been found to result in very high level expression of antibodies upon incorporation of variable and constant region genes, transfection in CHO cells, followed by selection in G418 containing medium and methotrexate amplification. Of course, any expression vector which is capable of eliciting expression in eukaryotic cells may be used in the present invention. Examples of suitable vectors include, but are not limited to plasmids pcDNA3, pHCMV/Zeo, pCR3.1, pEF1/His,

pIND/GS, pRc/HCMV2, pSV40/Zeo2, pTRACER-HCMV, pUB6/V5-His, pVAX1, and pZeoSV2 (available from Invitrogen, San Diego, CA), and plasmid pCI (available from Promega, Madison, WI). In general, screening large numbers of transformed cells for those which express suitably high levels if immunoglobulin heavy and light chains is routine experimentation which can be carried out, for example, by robotic systems. Vector systems are also taught in U.S. Pat. Nos. 5,736,137 and 5,658,570, each of which is incorporated by reference in its entirety herein. This system provides for high expression levels, *e.g.*, > 30 pg/cell/day. Other exemplary vector systems are disclosed *e.g.*, in U.S. Patent 6,413,777.

[0198] In other preferred embodiments the antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention may be expressed using polycistronic constructs such as those disclosed in United States Patent Application Publication No. 2003-0157641 A1, filed November 18, 2002 and incorporated herein in its entirety. In these novel expression systems, multiple gene products of interest such as heavy and light chains of antibodies may be produced from a single polycistronic construct. These systems advantageously use an internal ribosome entry site (IRES) to provide relatively high levels of antibodies. Compatible IRES sequences are disclosed in U.S. Pat. No. 6,193,980 which is also incorporated herein. Those skilled in the art will appreciate that such expression systems may be used to effectively produce the full range of antibodies disclosed in the instant application.

[0199] More generally, once the vector or DNA sequence encoding a monomeric subunit of the antibody has been prepared, the expression vector may be introduced into an appropriate host cell. Introduction of the plasmid into the host cell can be accomplished by various techniques well known to those of skill in the art. These include, but are not limited to, transfection (including electrophoresis and electroporation), protoplast fusion, calcium phosphate precipitation, cell fusion with enveloped DNA, microinjection, and infection with intact virus. *See*, Ridgway, A. A. G. "*Mammalian Expression Vectors*" Vectors, Rodriguez and Denhardt, Eds., Butterworths, Boston, Mass., Chapter 24.2, pp. 470-472 (1988). Typically, plasmid introduction into the host is via electroporation. The host cells harboring the expression construct are grown under conditions appropriate to the production of the light chains and heavy chains, and assayed for heavy and/or light chain protein synthesis. Exemplary assay techniques include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), or fluorescence-activated cell sorter analysis (FACS), immunohistochemistry and the like.

[0200] The expression vector is transferred to a host cell by conventional techniques and the transfected cells are then cultured by conventional techniques to produce an antibody for use in the methods described herein. Thus, the invention includes host cells containing a polynucleotide encoding an antibody of the invention, or a heavy or light chain thereof, operably linked to a heterologous promoter. In preferred embodiments for the expression of double-chained antibodies, vectors encoding both the heavy and light chains may be co-expressed in the host cell for expression of the entire immunoglobulin molecule, as detailed below.

[0201] The present invention furthermore relates to host cells transformed with a polynucleotide or vector of the invention. Said host cell may be a prokaryotic or eukaryotic cell. The polynucleotide or vector of the invention which is present in the host cell may either be integrated into the genome of the host cell or it may be maintained extrachromosomally. The host cell can be any prokaryotic or eukaryotic cell, such as a bacterial, insect, fungal, plant, animal or human cell. Preferred fungal cells are, for example, those of the genus *Saccharomyces*, in particular those of the species *S. cerevisiae*. The term "prokaryotic" is meant to include all bacteria which can be transformed or transfected with a DNA or RNA molecules for the expression of an antibody of the invention or the corresponding immu-noglobulin chains. Prokaryotic hosts may include gram negative as well as gram positive bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells, most preferably HEK 293, NSO and CHO cells. Depending upon the host employed in a recombinant production procedure, the antibodies or immunoglobulin chains encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Antibodies of the invention or the corresponding immunoglobulin chains may also include an initial methionine amino acid residue. A polynucleotide of the invention can be used to transform or transfect the host using any of the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, *e.g.*, mammalian cells and bacteria are well-known in the art (Sambrook, Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1989). The genetic constructs and methods described therein can be utilized for expression of the antibody of the invention or the corresponding immunoglobulin chains in eukaryotic or prokaryotic hosts. In general, expression vectors containing

promoter sequences which facilitate the efficient transcription of the inserted polynucleotide are used in connection with the host. The expression vector typically contains an origin of replication, a promoter, and a terminator, as well as specific genes which are capable of providing phenotypic selection of the transformed cells. Suitable source cells for the DNA sequences and host cells for immunoglobulin expression and secretion can be obtained from a number of sources, such as the American Type Culture Collection ("Catalogue of Cell Lines and Hybridomas," Fifth edition (1985) Rockville, Maryland, U.S.A., which is incorporated herein by reference). Furthermore, transgenic animals, preferably mammals, comprising cells of the invention may be used for the large scale production of the antibody of the invention.

[0202] Thus, in a further embodiment, the present invention relates to a method for the production of a disorder-associated protein specific binding molecule, *e.g.*, an antibody or a binding fragment or immunoglobulin chain(s) thereof, said method comprising

(a) culturing a cell as described above; and

(b) isolating said antigen, binding molecule, antibody or binding fragment or immunoglobulin chain(s) thereof from the culture.

[0203] The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention, can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer Verlag, N.Y. (1982). The antibody or its corresponding immunoglobulin chain(s) of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and purification of the, *e.g.*, recombinantly expressed antibodies or immunoglobulin chains of the invention may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, *e.g.*, against the constant region of the antibody of the invention. It will be apparent to those skilled in the art that the antibodies of the invention can be further coupled to other moieties for, *e.g.*, drug targeting and imaging applications. Such coupling may be conducted chemically after expression of the antibody or antigen to site of attachment or the coupling product may be engineered into the antibody or antigen of the invention at the DNA level. The DNAs are

then expressed in a suitable host system, and the expressed proteins are collected and renatured, if necessary.

[0204] Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the antibodies may then be used therapeutically (including extracorporally) or in developing and performing assay procedures.

[0205] The host cell may be co-transfected with two expression vectors of the invention, the first vector encoding a heavy chain derived polypeptide and the second vector encoding a light chain derived polypeptide. The two vectors may contain identical selectable markers which enable equal expression of heavy and light chain polypeptides. Alternatively, a single vector may be used which encodes both heavy and light chain polypeptides. In such situations, the light chain is advantageously placed before the heavy chain to avoid an excess of toxic free heavy chain (Proudfoot, *Nature 322*:52 (1986); Kohler, *Proc. Natl. Acad. Sci. USA 77*:2197 (1980)). The coding sequences for the heavy and light chains may comprise cDNA or genomic DNA.

[0206] As used herein, "host cells" refers to cells which harbor vectors constructed using recombinant DNA techniques and encoding at least one heterologous gene. In descriptions of processes for isolation of antibodies from recombinant hosts, the terms "cell" and "cell culture" are used interchangeably to denote the source of antibody unless it is clearly specified otherwise. In other words, recovery of polypeptide from the "cells" may mean either from spun down whole cells, or from the cell culture containing both the medium and the suspended cells.

[0207] A variety of host-expression vector systems may be utilized to express antibody molecules for use in the methods described herein. Such host-expression systems represent vehicles by which the coding sequences of interest may be produced and subsequently purified, but also represent cells which may, when transformed or transfected with the appropriate nucleotide coding sequences, express an antibody molecule of the invention *in situ*. These include but are not limited to microorganisms such as bacteria (*e.g.*, *E. coli*, *B. subtilis*) transformed with recombinant bacteriophage DNA, plasmid DNA or cosmid DNA expression vectors containing antibody coding sequences; yeast (*e.g.*, *Saccharomyces*, *Pichia*) transformed with recombinant yeast expression vectors containing antibody coding sequences; insect cell systems infected with recombinant virus expression vectors (*e.g.*,

baculovirus) containing antibody coding sequences; plant cell systems infected with recombinant virus expression vectors (*e.g.*, cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (*e.g.*, Ti plasmid) containing antibody coding sequences; or mammalian cell systems (*e.g.*, COS, CHO, BLK, 293, 3T3 cells) harboring recombinant expression constructs containing promoters derived from the genome of mammalian cells (*e.g.*, metallothionein promoter) or from mammalian viruses (*e.g.*, the adenovirus late promoter; the vaccinia virus 7.5K promoter). Preferably, bacterial cells such as *Escherichia coli*, and more preferably, eukaryotic cells, especially for the expression of whole recombinant antibody molecule, are used for the expression of a recombinant antibody molecule. For example, mammalian cells such as Chinese hamster ovary cells (CHO), in conjunction with a vector such as the major intermediate early gene promoter element from human cytomegalovirus is an effective expression system for antibodies (Foecking *et al.*, *Gene 45*:101 (1986); Cockett *et al.*, *Bio/Technology 8*:2 (1990)).

[0208] The host cell line used for protein expression is often of mammalian origin; those skilled in the art are credited with ability to preferentially determine particular host cell lines which are best suited for the desired gene product to be expressed therein. Exemplary host cell lines include, but are not limited to, CHO (Chinese Hamster Ovary), DG44 and DUXB11 (Chinese Hamster Ovary lines, DHFR minus), HELA (human cervical carcinoma), CVI (monkey kidney line), COS (a derivative of CVI with SV40 T antigen), VERY, BHK (baby hamster kidney), MDCK, 293, WI38, R1610 (Chinese hamster fibroblast) BALBC/3T3 (mouse fibroblast), HAK (hamster kidney line), SP2/O (mouse myeloma), P3x63-Ag3.653 (mouse myeloma), BFA-1c1BPT (bovine endothelial cells), RAJI (human lymphocyte) and 293 (human kidney). CHO cells are particularly preferred. Host cell lines are typically available from commercial services, the American Tissue Culture Collection or from published literature.

[0209] In addition, a host cell strain may be chosen which modulates the expression of the inserted sequences, or modifies and processes the gene product in the specific fashion desired. Such modifications (*e.g.*, glycosylation) and processing (*e.g.*, cleavage) of protein products may be important for the function of the protein. Different host cells have characteristic and specific mechanisms for the post-translational processing and modification of proteins and gene products. Appropriate cell lines or host systems can be chosen to ensure the correct modification and processing of the foreign protein expressed.

To this end, eukaryotic host cells which possess the cellular machinery for proper processing of the primary transcript, glycosylation, and phosphorylation of the gene product may be used.

[0210] For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express the antibody molecule may be engineered. Rather than using expression vectors which contain viral origins of replication, host cells can be transformed with DNA controlled by appropriate expression control elements (*e.g.*, promoter, enhancer, sequences, transcription terminators, polyadenylation sites, etc.), and a selectable marker. Following the introduction of the foreign DNA, engineered cells may be allowed to grow for 1-2 days in an enriched media, and then are switched to a selective media. The selectable marker in the recombinant plasmid confers resistance to the selection and allows cells to stably integrate the plasmid into their chromosomes and grow to form foci which in turn can be cloned and expanded into cell lines. This method may advantageously be used to engineer cell lines which stably express the antibody molecule.

[0211] A number of selection systems may be used, including but not limited to the herpes simplex virus thymidine kinase (Wigler *et al.*, *Cell 11*:223 (1977)), hypoxanthine-guanine phosphoribosyltransferase (Szybalska & Szybalski, *Proc. Natl. Acad. Sci. USA 48*:202 (1992)), and adenine phosphoribosyltransferase (Lowy *et al.*, *Cell 22*:817 1980) genes can be employed in tk-, hgprt- or aprt-cells, respectively. Also, anti-metabolite resistance can be used as the basis of selection for the following genes: dhfr, which confers resistance to methotrexate (Wigler *et al.*, *Natl. Acad. Sci. USA 77*:357 (1980); O'Hare *et al.*, *Proc. Natl. Acad. Sci. USA 78*:1527 (1981)); gpt, which confers resistance to mycophenolic acid (Mulligan & Berg, *Proc. Natl. Acad. Sci. USA 78*:2072 (1981)); neo, which confers resistance to the aminoglycoside G-418 *Clinical Pharmacy 12*:488-505; Wu and Wu, *Biotherapy 3*:87-95 (1991); Tolstoshev, *Ann. Rev. Pharmacol. Toxicol. 32*:573-596 (1993); Mulligan, *Science 260*:926-932 (1993); and Morgan and Anderson, *Ann. Rev. Biochem. 62*:191-217 (1993); *TIB TECH 11(5)*:155-215 (May, 1993); and hygro, which confers resistance to hygromycin (Santerre *et al.*, *Gene 30*:147 (1984). Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel *et al.* (eds.), *Current Protocols in Molecular Biology*, John Wiley & Sons, NY (1993); Kriegler, *Gene Transfer and Expression*, A Laboratory Manual, Stockton Press, NY (1990); and in Chapters 12 and 13, Dracopoli *et al.* (eds), *Current Protocols in Human*

Genetics, John Wiley & Sons, NY (1994); Colberre-Garapin *et al.*, *J. Mol. Biol. 150*:1 (1981), which are incorporated by reference herein in their entireties.

[0212] The expression levels of an antibody molecule can be increased by vector amplification (for a review, *see* Bebbington and Hentschel, *The use of* vectors *based on gene amplification for the expression of cloned genes in mammalian cells in DNA cloning*, Academic Press, New York, Vol. 3. (1987)). When a marker in the vector system expressing antibody is amplifiable, increase in the level of inhibitor present in culture of host cell will increase the number of copies of the marker gene. Since the amplified region is associated with the antibody gene, production of the antibody will also increase (Crouse *et al.*, *Mol. Cell. Biol. 3*:257 (1983)).

[0213] *In vitro* production allows scale-up to give large amounts of the desired polypeptides. Techniques for mammalian cell cultivation under tissue culture conditions are known in the art and include homogeneous suspension culture, *e.g.* in an airlift reactor or in a continuous stirrer reactor, or immobilized or entrapped cell culture, *e.g.* in hollow fibers, microcapsules, on agarose microbeads or ceramic cartridges. If necessary and/or desired, the solutions of polypeptides can be purified by the customary chromatography methods, for example gel filtration, ion-exchange chromatography, chromatography over DEAE-cellulose or (immuno-)affinity chromatography, *e.g.*, after preferential biosynthesis of a synthetic hinge region polypeptide or prior to or subsequent to the HIC chromatography step described herein.

[0214] Genes encoding antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can also be expressed non-mammalian cells such as bacteria or insect or yeast or plant cells. Bacteria which readily take up nucleic acids include members of the enterobacteriaceae, such as strains of *Escherichia coli* or *Salmonella*; Bacillaceae, such as *Bacillus subtilis*; *Pneumococcus*; *Streptococcus*, and *Haemophilus influenzae*. It will further be appreciated that, when expressed in bacteria, the heterologous polypeptides typically become part of inclusion bodies. The heterologous polypeptides must be isolated, purified and then assembled into functional molecules. Where tetravalent forms of antibodies are desired, the subunits will then self-assemble into tetravalent antibodies (WO02/096948A2).

[0215] In bacterial systems, a number of expression vectors may be advantageously selected depending upon the use intended for the antibody molecule being expressed. For example, when a large quantity of such a protein is to be produced, for the generation of

pharmaceutical compositions of an antibody molecule, vectors which direct the expression of high levels of fusion protein products that are readily purified may be desirable. Such vectors include, but are not limited, to the *E. coli* expression vector pUR278 (Ruther *et al.*, *EMBO J. 2*:1791 (1983)), in which the antibody coding sequence may be ligated individually into the vector in frame with the lacZ coding region so that a fusion protein is produced; pIN vectors (Inouye & Inouye, *Nucleic Acids Res. 13*:3101-3109 (1985); Van Heeke & Schuster, *J. Biol. Chem. 24*:5503-5509 (1989)); and the like. pGEX vectors may also be used to express foreign polypeptides as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption and binding to a matrix glutathione-agarose beads followed by elution in the presence of free glutathione. The pGEX vectors are designed to include thrombin or factor Xa protease cleavage sites so that the cloned target gene product can be released from the GST moiety.

[0216] In addition to prokaryotes, eukaryotic microbes may also be used. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among eukaryotic microorganisms although a number of other strains are commonly available, *e.g.*, *Pichia pastoris*.

[0217] For expression in *Saccharomyces*, the plasmid YRp7, for example, (Stinchcomb *et al.*, Nature *282*:39 (1979); Kingsman *et al.*, *Gene 7*:141 (1979); Tschemper *et al.*, *Gene 10*:157 (1980)) is commonly used. This plasmid already contains the TRP1 gene which provides a selection marker for a mutant strain of yeast lacking the ability to grow in tryptophan, for example ATCC No. 44076 or PEP4-1 (Jones, *Genetics 85*:12 (1977)). The presence of the trpl lesion as a characteristic of the yeast host cell genome then provides an effective environment for detecting transformation by growth in the absence of tryptophan.

[0218] In an insect system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is typically used as a vector to express foreign genes. The virus grows in Spodoptera *frugiperda* cells. The antibody coding sequence may be cloned individually into non-essential regions (for example the polyhedrin gene) of the virus and placed under control of an AcNPV promoter (for example the polyhedrin promoter).

[0219] Once an antibody molecule of the invention has been recombinantly expressed, it may be purified by any method known in the art for purification of an immunoglobulin molecule, for example, by chromatography (*e.g.*, ion exchange, affinity, particularly by affinity for the specific antigen after Protein A, and sizing column chromatography),

centrifugation, differential solubility, or by any other standard technique for the purification of proteins. Alternatively, a preferred method for increasing the affinity of antibodies of the invention is disclosed in US 2002 0123057 A1.

VI. Fusion Proteins and Conjugates

[0220] The antibodies of the present invention can comprise a further domain, said domain being linked by covalent or non-covalent bonds. The linkage can be based on genetic fusion according to the methods known in the art and described above or can be performed by, *e.g.*, chemical cross-linking as described in, *e.g.*, international application WO94/04686. The additional domain present in the fusion protein comprising the antibody of the invention may preferably be linked by a flexible linker, advantageously a polypeptide linker, wherein said polypeptide linker comprises plural, hydrophilic, peptide-bonded amino acids of a length sufficient to span the distance between the C-terminal end of said further domain and the N-terminal end of the antibody of the invention or vice versa. The therapeutically or diagnostically active agent can be coupled to the antibody of the invention or an antigen-binding fragment thereof by various means. This includes, for example, single-chain fusion proteins comprising the variable regions of the antibody of the invention coupled by covalent methods, such as peptide linkages, to the therapeutically or diagnostically active agent. Further examples include molecules which comprise at least an antigen-binding fragment coupled to additional molecules covalently or non-covalently include those in the following non-limiting illustrative list. Traunecker, Int. J. Cancer Surp. SuDP 7 (1992), 51-52, describe the bispecific reagent janusin in which the Fv region directed to CD3 is coupled to soluble CD4 or to other ligands such as OVCA and IL-7. Similarly, the variable regions of the antibody of the invention can be constructed into Fv molecules and coupled to alternative ligands such as those illustrated in the cited article. Higgins, J. Infect Disease 166 (1992), 198-202, described a hetero-conjugate antibody composed of OKT3 cross-linked to an antibody directed to a specific sequence in the V3 region of GP120. Such hetero-conjugate antibodies can also be constructed using at least the variable regions contained in the antibody of the invention methods. Additional examples of specific antibodies include those described by Fanger, Cancer Treat. Res. 68 (1993), 181-194 and by Fanger, Crit. Rev. Immunol. 12 (1992), 101-124.

[0221] In a further embodiment of the present invention, the binding molecule, antibody, immunoglobulin chain or a binding fragment thereof or the antigen is detectably labeled. Labeling agents can be coupled either directly or indirectly to the antibodies or antigens of the invention. One example of indirect coupling is by use of a spacer moiety.

[0222] Hence, the biological activity of the binding molecules, *e.g.* antibodies identified here suggests that they have sufficient affinity to make them potential candidates for drug localization to cells expressing the appropriate surface structures of the diseased cell and tissue, respectively. This targeting and binding to cells could be useful for the delivery of therapeutically or diagnostically active agents and gene therapy/gene delivery. Molecules/particles with an antibody of the invention would bind specifically to cells/tissues expressing the variant form of the pathological protein, and therefore could have diagnostic and therapeutic use. Thus, the binding molecule, *e.g.*, antibody or antigen binding fragment thereof of the present invention can be labeled (*e.g.*, fluorescent, radioactive, enzyme, nuclear magnetic, heavy metal) and used to detect specific targets *in vivo* or *in vitro* including "immunochemistry" like assays *in vitro*. *In vivo* they could be used in a manner similar to nuclear medicine imaging techniques to detect tissues, cells, or other material expressing the neoepitope. Thus, in a further embodiment the present invention relates to the use of a binding molecule or an antibody of the present invention or binding fragment thereof for the preparation of a composition for *in vivo* detection of or targeting a therapeutic and/or diagnostic agent to a disorder-associated protein in the brain, detecting, suppressing formation of or reducing pathological protein aggregates or conformations in a subject, for improving cognition or slowing or reversing cognitive decline associated with diseases, or for extra-corporal extraction of pathological compounds or their precursors from body fluids.

[0223] In certain embodiments, antibody polypeptide comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, a single-chain fv antibody fragment of the invention may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0224] An antibody polypeptide of the invention may comprise, consist essentially of, or consist of a fusion protein. Fusion proteins are chimeric molecules which comprise, for example, an immunoglobulin antigen-binding domain with at least one target binding site, and at least one heterologous portion, *i.e.*, a portion with which it is not naturally linked in

nature. The amino acid sequences may normally exist in separate proteins that are brought together in the fusion polypeptide or they may normally exist in the same protein but are placed in a new arrangement in the fusion polypeptide. Fusion proteins may be created, for example, by chemical synthesis, or by creating and translating a polynucleotide in which the peptide regions are encoded in the desired relationship.

[0225] The term "heterologous" as applied to a polynucleotide or a polypeptide, means that the polynucleotide or polypeptide is derived from a distinct entity from that of the rest of the entity to which it is being compared. For instance, as used herein, a "heterologous polypeptide" to be fused to an antibody, or an antigen-binding fragment, variant, or analog thereof is derived from a non-immunoglobulin polypeptide of the same species, or an immunoglobulin or non-immunoglobulin polypeptide of a different species.

[0226] As discussed in more detail elsewhere herein, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention may further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalent and non-covalent conjugations) to polypeptides or other compositions. For example, antibodies may be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, radionuclides, or toxins. *See*, *e.g.*, PCT publications WO 92/08495; WO 91/14438; WO 89/12624; U.S. Patent No. 5,314,995; and EP 396,387.

[0227] Antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can be composed of amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres, and may contain amino acids other than the 20 gene-encoded amino acids. Antibodies may be modified by natural processes, such as posttranslational processing, or by chemical modification techniques which are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature. Modifications can occur anywhere in the antibody, including the peptide backbone, the amino acid side-chains and the amino or carboxyl termini, or on moieties such as carbohydrates. It will be appreciated that the same type of modification may be present in the same or varying degrees at several sites in a given antibody. Also, a given antibody may contain many types of modifications. Antibodies may be branched, for example, as a result of ubiquitination, and they may be cyclic, with or without branching. Cyclic, branched, and branched cyclic antibodies may result from posttranslation natural processes or may be made by synthetic methods.

Modifications include acetylation, acylation, ADP-ribosylation, amidation, covalent attachment of flavin, covalent attachment of a heme moiety, covalent attachment of a nucleotide or nucleotide derivative, covalent attachment of a lipid or lipid derivative, covalent attachment of phosphotidylinositol, cross-linking, cyclization, disulfide bond formation, demethylation, formation of covalent cross-links, formation of cysteine, formation of pyroglutamate, formylation, gamma-carboxylation, glycosylation, GPI anchor formation, hydroxylation, iodination, methylation, myristoylation, oxidation, pegylation, proteolytic processing, phosphorylation, prenylation, racemization, selenoylation, sulfation, transfer-RNA mediated addition of amino acids to proteins such as arginylation, and ubiquitination. (*See, e.g.*, *Proteins - Structure And Molecular Properties*, T. E. Creighton, W. H. Freeman and Company, New York 2nd Ed., (1993); *Posttranslational Covalent Modification Of Proteins*, B. C. Johnson, Ed., Academic Press, New York, pgs. 1-12 (1983); Seifter *et al.*, *Meth Enzymol 182*:626-646 (1990); Rattan *et al.*, *Ann NY Acad Sci 663*:48-62 (1992)).

[0228] The present invention also provides for fusion proteins comprising an antibody, or antigen-binding fragment, variant, or derivative thereof, and a heterologous polypeptide. In one embodiment, a fusion protein of the invention comprises, consists essentially of, or consists of, a polypeptide having the amino acid sequence of any one or more of the VH regions of an antibody of the invention or the amino acid sequence of any one or more of the VL regions of an antibody of the invention or fragments or variants thereof, and a heterologous polypeptide sequence. In another embodiment, a fusion protein for use in the diagnostic and treatment methods disclosed herein comprises, consists essentially of, or consists of a polypeptide having the amino acid sequence of any one, two, three of the VH-CDRs of an antibody, or fragments, variants, or derivatives thereof, or the amino acid sequence of any one, two, three of the VL-CDRs of an antibody, or fragments, variants, or derivatives thereof, and a heterologous polypeptide sequence. In one embodiment, the fusion protein comprises a polypeptide having the amino acid sequence of a VH-CDR3 of an antibody of the present invention, or fragment, derivative, or variant thereof, and a heterologous polypeptide sequence, which fusion protein specifically binds to at least one neoepitope of a disorder-associated protein. In another embodiment, a fusion protein comprises a polypeptide having the amino acid sequence of at least one VH region of an antibody of the invention and the amino acid sequence of at least one VL region of an antibody of the invention or fragments, derivatives or variants thereof, and a heterologous

polypeptide sequence. Preferably, the VH and VL regions of the fusion protein correspond to a single source antibody (or scFv or Fab fragment) which specifically binds at least one neoepitope of a disorder-associated protein. In yet another embodiment, a fusion protein for use in the diagnostic and treatment methods disclosed herein comprises a polypeptide having the amino acid sequence of any one, two, three or more of the VH CDRs of an antibody and the amino acid sequence of any one, two, three or more of the VL CDRs of an antibody, or fragments or variants thereof, and a heterologous polypeptide sequence. Preferably, two, three, four, five, six, or more of the VH-CDR(s) or VL-CDR(s) correspond to single source antibody (or scFv or Fab fragment) of the invention. Nucleic acid molecules encoding these fusion proteins are also encompassed by the invention.

[0229] Exemplary fusion proteins reported in the literature include fusions of the T cell receptor (Gascoigne *et al.*, *Proc. Natl. Acad. Sci. USA 84*:2936-2940 (1987)); CD4 (Capon *et al.*, *Nature 337*:525-531 (1989); Traunecker *et al.*, *Nature 339*:68-70 (1989); Zettmeissl *et al.*, *DNA Cell* Biol. *USA 9*:347-353 (1990); and Byrn *et al.*, *Nature 344*:667-670 (1990)); L-selectin (homing receptor) (Watson *et al.*, *J. Cell. Biol. 110*:2221-2229 (1990); and Watson *et al.*, *Nature 349*:164-167 (1991)); CD44 (Aruffo *et al.*, *Cell 61*:1303-1313 (1990)); CD28 and B7 (Linsley *et al.*, *J. Exp. Med. 173*:721-730 (1991)); CTLA-4 (Lisley *et al.*, *J. Exp. Med. 174*:561-569 (1991)); CD22 (Stamenkovic *et al.*, *Cell 66*:1133-1144 (1991)); TNF receptor (Ashkenazi *et al.*, *Proc. Natl. Acad. Sci. USA 88*:10535-10539 (1991); Lesslauer *et al.*, *Eur. J. Immunol. 27*:2883-2886 (1991); and Peppel *et al.*, *J. Exp. Med. 174*:1483-1489 (1991)); and IgE receptor a (Ridgway and Gorman, *J. Cell. Biol. Vol. 115*, Abstract No. 1448 (1991)).

[0230] As discussed elsewhere herein, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention may be fused to heterologous polypeptides to increase the *in vivo* half life of the polypeptides or for use in immunoassays using methods known in the art. For example, in one embodiment, PEG can be conjugated to the antibodies of the invention to increase their half-life *in vivo*. Leong, S.R., *et al.*, *Cytokine 16*:106 (2001); *Adv. in Drug Deliv. Rev. 54*:531 (2002); or Weir *et al.*, *Biochem. Soc. Transactions 30*:512 (2002).

[0231] Moreover, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can be fused to marker sequences, such as a peptide to facilitate their purification or detection. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., 9259 Eton

Avenue, Chatsworth, Calif., 91311), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA 86*:821-824 (1989), for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the "HA" tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, *Cell 37*:767 (1984)) and the "flag" tag.

[0232] Fusion proteins can be prepared using methods that are well known in the art (see for example US Patent Nos. 5,116,964 and 5,225,538). The precise site at which the fusion is made may be selected empirically to optimize the secretion or binding characteristics of the fusion protein. DNA encoding the fusion protein is then transfected into a host cell for expression.

[0233] Antibodies of the present invention may be used in non-conjugated form or may be conjugated to at least one of a variety of molecules, *e.g.*, to improve the therapeutic properties of the molecule, to facilitate target detection, or for imaging or therapy of the patient. Antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention can be labeled or conjugated either before or after purification, when purification is performed.

[0234] In particular, antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0235] Conjugates that are immunotoxins including conventional antibodies have been widely described in the art. The toxins may be coupled to the antibodies by conventional coupling techniques or immunotoxins containing protein toxin portions can be produced as fusion proteins. The antibodies of the present invention can be used in a corresponding way to obtain such immunotoxins. Illustrative of such immunotoxins are those described by Byers, Seminars Cell. Biol. 2 (1991), 59-70 and by Fanger, Immunol. Today 12 (1991), 51-54.

[0236] The above described fusion protein may further comprise a cleavable linker or cleavage site for proteinases. These spacer moieties, in turn, can be either insoluble or soluble (Diener *et al.*, Science 231 (1986), 148) and can be selected to enable drug release from the antigen at the target site. Examples of therapeutic agents which can be coupled to the antibodies and antigens of the present invention for immunotherapy are drugs,

radioisotopes, lectins, and toxins. The drugs with which can be conjugated to the antibodies and antigens of the present invention include compounds which are classically referred to as drugs such as mitomycin C, daunorubicin, and vinblastine. In using radioisotopically conjugated antibodies or antigens of the invention for, *e.g.*, immunotherapy, certain isotopes may be more preferable than others depending on such factors as leukocyte distribution as well as stability and emission. Depending on the autoimmune response, some emitters may be preferable to others. In general, α and ß particle emitting radioisotopes are preferred in immunotherapy. Preferred are short range, high energy a emitters such as ^{212}Bi. Examples of radioisotopes which can be bound to the antibodies or antigens of the invention for therapeutic purposes include, but are not limited to ^{125}I, ^{131}I, ^{90}Y, ^{67}Cu, ^{64}Cu, ^{212}Bi, ^{212}At, ^{211}Pb, ^{47}Sc, ^{109}Pd and ^{188}Re. Other therapeutic agents which can be coupled to the binding molecule, *e.g.*, antibody or antigen binding fragment thereof of the invention, as well as ex vivo and *in vivo* therapeutic protocols, are known, or can be easily ascertained, by those of ordinary skill in the art. Wherever appropriate the person skilled in the art may use a polynucleotide of the invention encoding any one of the above described antibodies, antigens or the corresponding vectors instead of the proteinaeous material itself.

[0237] Those skilled in the art will appreciate that conjugates may also be assembled using a variety of techniques depending on the selected agent to be conjugated. For example, conjugates with biotin are prepared *e.g.* by reacting a binding polypeptide with an activated ester of biotin such as the biotin N-hydroxysuccinimide ester. Similarly, conjugates with a fluorescent marker may be prepared in the presence of a coupling agent, *e.g.* those listed herein, or by reaction with an isothiocyanate, preferably fluorescein-isothiocyanate. Conjugates of the antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention are prepared in an analogous manner.

[0238] The present invention further encompasses antibodies, or antigen-binding fragments, variants, or derivatives thereof of the invention conjugated to a diagnostic or therapeutic agent. The antibodies can be used diagnostically to, for example, monitor the development or progression of a neurological disease as part of a clinical testing procedure to, *e.g.*, determine the efficacy of a given treatment and/or prevention regimen. Detection can be facilitated by coupling the antibody, or antigen-binding fragment, variant, or derivative thereof to a detectable substance. Examples of detectable substances include

various enzymes, prosthetic groups, fluorescent materials, luminescent materials, bioluminescent materials, radioactive materials, positron emitting metals using various positron emission tomographies, and nonradioactive paramagnetic metal ions. *See, e.g.*, U.S. Pat. No. 4,741,900 for metal ions which can be conjugated to antibodies for use as diagnostics according to the present invention. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; examples of bioluminescent materials include luciferase, luciferin, and aequorin; and examples of suitable radioactive material include ^{125}I, ^{131}I, ^{111}In or ^{99}Tc.

[0239] An antibody, or antigen-binding fragment, variant, or derivative thereof also can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antibody is then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, theromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0240] One of the ways in which an antibody, or antigen-binding fragment, variant, or derivative thereof can be detectably labeled is by linking the same to an enzyme and using the linked product in an enzyme immunoassay (EIA) (Voller, A., "The Enzyme Linked Immunosorbent Assay (ELISA)" Microbiological Associates Quarterly Publication, Walkersville, Md., *Diagnostic Horizons 2*:1-7 (1978)); Voller *et al.*, *J. Clin. Pathol. 31*:507-520 (1978); Butler, J. E., *Meth.* Enzymol. *73*:482-523 (1981); Maggio, E. (ed.), *Enzyme Immunoassay*, CRC Press, Boca Raton, Fla., (1980); Ishikawa, E. *et al.*, (eds.), *Enzyme Immunoassay*, Kgaku Shoin, Tokyo (1981). The enzyme, which is bound to the antibody will react with an appropriate substrate, preferably a chromogenic substrate, in such a manner as to produce a chemical moiety which can be detected, for example, by spectrophotometric, fluorimetric or by visual means. Enzymes which can be used to detectably label the antibody include, but are not limited to, malate dehydrogenase, staphylococcal nuclease, delta-5-steroid isomerase, yeast alcohol dehydrogenase, alpha-glycerophosphate, dehydrogenase, triose phosphate isomerase, horseradish peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, beta-galactosidase,

ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase and acetylcholinesterase. Additionally, the detection can be accomplished by colorimetric methods which employ a chromogenic substrate for the enzyme. Detection may also be accomplished by visual comparison of the extent of enzymatic reaction of a substrate in comparison with similarly prepared standards.

[0241] Detection may also be accomplished using any of a variety of other immunoassays. For example, by radioactively labeling the antibody, or antigen-binding fragment, variant, or derivative thereof, it is possible to detect the antibody through the use of a radioimmunoassay (RIA) (*see*, for example, Weintraub, B., *Principles of Radioimmunoassays, Seventh Training Course on Radioligand Assay Techniques*, The Endocrine Society, (March, 1986)), which is incorporated by reference herein). The radioactive isotope can be detected by means including, but not limited to, a gamma counter, a scintillation counter, or autoradiography.

[0242] An antibody, or antigen-binding fragment, variant, or derivative thereof can also be detectably labeled using fluorescence emitting metals such as 152Eu, or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

[0243] Techniques for conjugating various moieties to an antibody, or antigen-binding fragment, variant, or derivative thereof are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.* (eds.), Marcel Dekker, Inc., pp. 623-53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev. 62*:119-58 (1982).

[0244] In certain embodiments, a moiety that enhances the stability or efficacy of a binding molecule, *e.g.*, a binding polypeptide, *e.g.*, an antibody or immunospecific fragment thereof can be conjugated. For example, in one embodiment, PEG can be conjugated to the

binding molecules of the invention to increase their half-life *in vivo*. Leong, S.R., *et al.*, *Cytokine 16*:106 (2001); *Adv. in Drug Deliv. Rev. 54*:531 (2002); or Weir *et al.*, *Biochem. Soc. Transactions 30*:512 (2002).

VII. Compositions and Methods of Use

[0245] Moreover, the present invention relates to compositions comprising the aforementioned binding molecule, *e.g.*, antibody or antigen binding fragment thereof of the present invention or chemical derivatives thereof, or the polynucleotide, vector or cell of the invention. The composition of the present invention may further comprise a pharmaceutically acceptable carrier. The term "chemical derivative" describes a molecule that contains additional chemical moieties that are not normally a part of the base molecule. Such moieties may improve the solubility, half-life, absorption, etc. of the base molecule. Alternatively the moieties may attenuate undesirable side effects of the base molecule or decrease the toxicity of the base molecule. Furthermore, the pharmaceutical composition of the present invention may comprise further agents such as interleukins or interferons depending on the intended use of the pharmaceutical composition. For example, for use in the treatment of Alzheimer's disease the additional agent may selected from the group consisting of small organic molecules, anti-Abeta antibodies, and combinations thereof. Hence, in a particular preferred embodiment the present invention relates to the use of the binding molecule, *e.g.*, antibody or antigen binding fragment thereof of the present invention or of a binding molecule having substantially the same binding specificities of any one thereof, the polynucleotide, the vector or the cell of the present invention for the preparation of a pharmaceutical or diagnostic composition for treating or preventing the progression of Alzheimer's disease; for the amelioration of symptoms associated with Alzheimer's disease; for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's disease. Said pharmaceutical composition can be designed to be administered intravenously, intramuscularly, subcutaneously, intraperitoneally, intranasally, parenterally or as an aerosol; see also *infra*.

[0246] Hence, in one embodiment the present invention relates to a method of treating a neurological disorder characterized by abnormal accumulation and/or deposition of a protein in the central nervous system, which method comprises administering to a subject in need thereof a therapeutically effective amount of any one of the afore-described binding

molecules, antibodies, antigens, polynucleotides, vectors or cells of the instant invention. The term "neurological disorder" includes but is not limited to Alzheimer's Disease, mild cognitive impairment, fronto-temporal dementia, Lewy-body disease, Parkinson's disease, Pick's disease, Binswanger's disease; congophilic amyloid angiopathy, cerebral amyloid angiopathy, Down's syndrome, multi-infarct dementia, Huntington's Disease, Creutzfeldt-Jakob Disease, AIDS dementia complex, depression, anxiety disorder, phobia, Bell's Palsy, epilepsy, encephalitis, multiple sclerosis; neuromuscular disorders, neurooncological disorders, brain tumors, neurovascular disorders including stroke, neuroimmunological disorders, neurootological disease, neurotrauma including spinal cord injury, pain including neuropathic pain, pediatric neurological and neuropsychiatric disorders, sleep disorders, Tourette syndrome, mild cognitive impairment, vascular dementia, multi-infarct dementia, cystic fibrosis, Gaucher's disease other movement disorders and disease of the central nervous system (CNS) in general. Unless stated otherwise, the terms neurodegenerative, neurological or neuropsychiatric are used interchangeably herein.

[0247] In the sense of the present invention, a method is disclosed for characterizing human antibodies for a large number of diseases and to also produce said antibodies subsequently in order to employ them diagnostically, therapeutically, or preventively in such patients whose immune system did not react with a corresponding immune response to the development of the pathology of the disease. In particular, this will have to be expected in diseases occurring at an advanced age because, as is known, the reactivity of the immune system continuously and significantly decreases as the age increases. In these cases, therapeutically or preventively active antibodies could compensate the age-related restrictions of the immune system with respect to blocking the enrichment of endogenous pathophysiological protein variants and could thus contribute to a better state of health at an advanced age. Thus, the medical uses of the present invention are particularly applicable for the above-described patient group, for example at the age of 60, 65, 70, 75, 80 or older and in principle concern all diseases manifesting themselves in form of derailment of any kind, like for example endoproteolysis, conformation alterations, alterations of the post-translational modifications, somatic mutations, or combinations thereof, phenotypically by means of developing pathophysiological variants of endogenous protein. In the sense of the present invention, pathophysiological variants are considered to be variants containing pathological neoepitopes that deviate from the physiology, see *supra*.

[0248] In particular, the therapeutic applications include tumor diseases, inflammatory diseases and diseases of the central nervous system, like Alzheimer's disease, Parkinson's disease, Pick's disease, Dementia with Lewy Bodies, Prion diseases including Creutzfeldt-Jakob disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration, frontotemporal degeneration with Parkinsonism liked to chromosome 17 Huntington's disease, frontotemporal dementia, cerebral amyloid angiopathy, mild cognitive impairment, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis Dutch type and Icelandic type, spinocerebellar ataxia and amyotrohic lateral sclerosis as well as glaucoma, inclusion body myositis, familial amyloid polyneuropathy and amyloidoses comprising fibrillary proteins derived from at least one of the following precursor proteins SAA (Serum-Amyloid-Protein A), AL (k or l-light chains of Immunoglobulins), AH (g1 Ig-heavy chains), ATTR (Transthyretin, Serum-Prealbumin), AApo-A-1 (Apolipoprotein A1), AApoA2 (Apolipoprotein A2), AGel (Gelsolin), ACys (Cystatin C), ALys (Lysozyme), AFib (Fibrinogen), Beta-amyloid (Amyloid precursor protein), Beta-amyloid2M (beta2-microglobulin), APrP (Prion protein), ACal (Procalcitonin), AIAPP (islet amyloid polypeptide); APro (Prolactin), AIns (Insulin); AMed (Lactadherin); Aker (Kerato-epithelin); ALac (Lactoferrin), Abri (AbriPP), ADan (ADanPP); or AANP (Atrial natriuretical peptide), (Skovronsky at al., Annu. Rev. Pathol. Mech. Dis. 2006; 1:151-70; Buxbaum, Curr Opin Rheumatol 2003; 16: 67-75.

[0249] A particular advantage of the therapeutic approach of the present invention lies in the fact that antibodies derived from B cells or B memory cells from a healthy preclinical or clinically unusually stable organism are, with a certain probability, capable of preventing a clinically manifest disease, or of diminishing the risk of the occurrence of a clinically manifest disease, or of delaying the moment of the occurrence of a clinically manifest disease. Typically, such antibodies also have already successfully gone through somatic maturation, *i.e.* the optimization with respect to selectivity and effectiveness in the high affinity binding to the target molecule by means of somatic variation of the variable regions of the antibody.

[0250] The knowledge that such cells *in vivo*, *e.g.* in a human, have not been activated by means of related or other physiological proteins or cell structures in the sense of an autoimmunological or allergic reaction is also of great medical importance since this signifies a considerably increased chance of successfully living through the clinical test

phases. So to speak, efficiency, acceptability and tolerability have already been demonstrated before the preclinical development of the prophylactic or therapeutic antibody in at least one human subject. It can thus be expected that, with a procedure according to the present invention, both the target structure-specific efficiency of an antibody as therapeutic agent and its decreased probability of side effects significantly increase its clinical probability of success.

[0251] From the foregoing, it is evident that the present invention encompasses any use of a disease specific binding molecule comprising at least one CDR of the above described antibody, in particular for diagnosing and/or treatment of a disorder related to Alzheimer's disease and Abeta deposition, respectively. Preferably, said binding molecule is an antibody of the present invention or an immunoglobulin chain thereof. In addition, the present invention relates to anti-idiotypic antibodies of any one of the mentioned antibodies described hereinbefore. These are antibodies or other binding molecules which bind to the unique antigenic peptide sequence located on an antibody's variable region near the antigen binding site.

[0252] In another embodiment the present invention relates to a diagnostic composition comprising any one of the above described binding molecules, antibodies, antigen-binding fragments, polynucleotides, vectors or cells of the invention and optionally suitable means for detection such as reagents conventionally used in immuno or nucleic acid based diagnostic methods. The antibodies of the invention are, for example, suited for use in immunoassays in which they can be utilized in liquid phase or bound to a solid phase carrier. Examples of immunoassays which can utilize the antibody of the invention are competitive and non-competitive immunoassays in either a direct or indirect format. Examples of such immunoassays are the radioimmunoassay (RIA), the sandwich (immunometric assay), flow cytometry and the Western blot assay. The antigens and antibodies of the invention can be bound to many different carriers and used to isolate cells specifically bound thereto. Examples of well known carriers include glass, polystyrene, polyvinyl chloride, polypropylene, polyethylene, polycarbonate, dextran, nylon, amyloses, natural and modified celluloses, polyacrylamides, agaroses, and magnetite. The nature of the carrier can be either soluble or insoluble for the purposes of the invention. There are many different labels and methods of labeling known to those of ordinary skill in the art. Examples of the types of labels which can be used in the present invention include enzymes, radioisotopes, colloidal metals, fluorescent compounds, chemiluminescent

compounds, and bioluminescent compounds; see also the embodiments discussed hereinabove.

[0253] By a further embodiment, the binding molecules, in particular antibodies of the present invention may also be used in a method for the diagnosis of a disorder in an individual by obtaining a body fluid sample from the tested individual which may be a blood sample, a lymph sample or any other body fluid sample and contacting the body fluid sample with an antibody of the instant invention under conditions enabling the formation of antibody-antigen complexes. The level of such complexes is then determined by methods known in the art, a level significantly higher than that formed in a control sample indicating the disease in the tested individual. In the same manner, the specific antigen bound by the antibodies of the invention may also be used. Thus, the present invention relates to an *in vitro* immunoassay comprising the binding molecule, *e.g.*, antibody or antigen binding fragment thereof of the invention.

[0254] In this context, the present invention also relates to means specifically designed for this purpose. For example, a protein- or antibody-based array may be used, which is for example loaded with either antigens derived from the mentioned disorder-associated protein and containing the neoepitope in order to detect autoantibodies which may be present in patients suffering from, *e.g.*, a neurological disorder, in particular Alzheimer's disease, or with antibodies or equivalent antigen-binding molecules of the present invention which specifically recognize any one of those proteins. For example, antigen microarray profiling of autoantibodies in rheumatoid arthritis has been reported by Hueber *et al.*, Arthritis Rheum. 52 (2005), 2645-2655. Design of microarray immunoassays is summarized in Kusnezow *et al.*, Mol. Cell Proteomics 5 (2006), 1681-1696. Accordingly, the present invention also relates to microarrays loaded with binding molecules or antigens identified in accordance with the present invention.

[0255] The present invention also provides a pharmaceutical and diagnostic, respectively, pack or kit comprising one or more containers filled with one or more of the above described ingredients, *e.g.* binding molecule, antibody or binding fragment thereof, antigen, polynucleotide, vector or cell of the present invention. Associated with such container(s) can be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration. In addition or alternatively the kit comprises reagents and/or instructions for use in appropriate

diagnostic assays. The composition, *e.g.* kit of the present invention is of course particularly suitable for the diagnosis, prevention and treatment of a disorder which is accompanied with the presence of a disorder-associated protein as defined above, especially amyloidosis, and in particular applicable for the treatment of Alzheimer's disease (AD).

[0256] The terms "treatment", "treating" and the like are used herein to generally mean obtaining a desired pharmacological and/or physiological effect. The effect may be prophylactic in terms of completely or partially preventing a disease or symptom thereof and/or may be therapeutic in terms of partially or completely curing a disease and/or adverse effect attributed to the disease. The term "treatment" as used herein covers any treatment of a disease in a mammal, particularly a human, and includes: (a) preventing the disease from occurring in a subject which may be predisposed to the disease but has not yet been diagnosed as having it; (b) inhibiting the disease, *e.g.* arresting its development; or (c) relieving the disease, *e.g.* causing regression of the disease.

[0257] Furthermore, the term "subject" or "patient" refers to a mammal, preferably a human, in need of treatment for a condition, disorder or disease.

[0258] The pharmaceutical compositions of the present invention can be formulated according to methods well known in the art; see for example Remington: The Science and Practice of Pharmacy (2000) by the University of Sciences in Philadelphia, ISBN 0-683-306472. Examples of suitable pharmaceutical carriers are well known in the art and include phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions etc. Compositions comprising such carriers can be formulated by well known conventional methods. These pharmaceutical compositions can be administered to the subject at a suitable dose. Administration of the suitable compositions may be effected by different ways, *e.g.*, by intravenous, intraperitoneal, subcutaneous, intramuscular, topical or intradermal administration. Aerosol formulations such as nasal spray formulations include purified aqueous or other solutions of the active agent with preservative agents and isotonic agents. Such formulations are preferably adjusted to a pH and isotonic state compatible with the nasal mucous membranes. Formulations for rectal or vaginal ad-ministration may be presented as a suppository with a suitable carrier.

[0259] Furthermore, whereas the present invention includes the now standard (though fortunately infrequent) procedure of drilling a small hole in the skull to administer a drug of

the present invention, in a preferred aspect, the binding molecule, especially antibody or antibody based drug of the present invention can cross the blood-brain barrier, which allows for intravenous or oral administration.

[0260] The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depends upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. A typical dose can be, for example, in the range of 0.001 to 1000 μg (or of nucleic acid for expression or for inhibition of expression in this range); however, doses below or above this exemplary range are envisioned, especially considering the aforementioned factors. Generally, the dosage can range, *e.g.*, from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg (e.g., 0.02 mg/kg, 0.25 mg/kg, 0.5 mg/kg, 0.75 mg/kg, 1mg/kg, 2 mg/kg, etc.), of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, preferably at least 1 mg/kg. Doses intermediate in the above ranges are also intended to be within the scope of the invention. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.. Progress can be monitored by periodic assessment. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may

also be present such as, for example, antimicrobials, anti-oxidants, chelating agents, and inert gases and the like. Furthermore, the pharmaceutical composition of the invention may comprise further agents such as dopamine or psychopharmacologic drugs, depending on the intended use of the pharmaceutical composition. Furthermore, the pharmaceutical composition may also be formulated as a vaccine, for example, if the pharmaceutical composition of the invention comprises an anti-Aβ antibody for passive immunization.

[0261] In addition, co-administration or sequential administration of other agents may be desirable. A therapeutically effective dose or amount refers to that amount of the active ingredient sufficient to ameliorate the symptoms or condition. Therapeutic efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, ED_{50} (the dose therapeutically effective in 50% of the population) and LD_{50} (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, LD_{50}/ED_{50}. Preferably, the therapeutic agent in the composition is present in an amount sufficient to restore normal behavior and/or cognitive properties in case of Alzheimer's disease.

[0262] The pharmaceutical compositions in accordance with the present invention can preferably be used for the treatment of neurological disorders including but not limited to Alzheimer's disease, Parkinson's disease, Pick's disease, Dementia with Lewy Bodies, Prion diseases including Creutzfeldt-Jakob disease, progressive supranuclear palsy, multiple system atrophy, corticobasal degeneration, frontotemporal degeneration with Parkinsonism liked to chromosome 17 Huntington's disease, frontotemporal dementia, cerebral amyloid angiopathy, mild cognitive impairment, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis Dutch type and Icelandic type, spinocerebellar ataxia, amyotrohic lateral sclerosis, Bell's Palsy, epilepsy, encephalitis, neuromuscular disorders, glaucoma, inclusion body myositis, familial amyloid polyneuropathy, amyloidoses comprising fibrillary proteins derived from at least one of the following precursor proteins SAA (Serum-Amyloid-Protein A), AL (k or l-light chains of Immunoglobulins), AH (g1 Ig-heavy chains), ATTR (Transthyretin, Serum-Prealbumin), AApo-A-1 (Apolipoprotein A1), AApoA2 (Apolipoprotein A2), AGel (Gelsolin), ACys (Cystatin C), ALys (Lysozyme), AFib (Fibrinogen), Beta-amyloid (Amyloid precursor protein), Beta-amyloid2M (beta2-microglobulin), APrP (Prion protein), ACal (Procalcitonin), AIAPP (islet amyloid polypeptide); APro (Prolactin), AIns (Insulin);

AMed (Lactadherin); Aker (Kerato-epithelin); ALac (Lactoferrin), Abri (AbriPP), ADan (ADanPP); or AANP (Atrial natriuretical peptide), (Skovronsky at al., Annu. Rev. Pathol. Mech. Dis. 2006; 1:151-70; Buxbaum, Curr Opin Rheumatol 2003; 16: 67-75, neuro-oncology, neuro-immunology, neuro-otology pain, pediatric neurology, phobia, affective disorders, sleep disorders, Tourette Syndrome, other movement disorders and disease of the central nervous system (CNS) in general.

[0263] These and other embodiments are disclosed and encompassed by the description and examples of the present invention. Further literature concerning any one of the materials, methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example the public database "Medline" may be utilized, which is hosted by the National Center for Biotechnology Information and/or the National Library of Medicine at the National Institutes of Health. Further databases and web addresses, such as those of the European Bioinformatics Institute (EBI), which is part of the European Molecular Biology Laboratory (EMBL) are known to the person skilled in the art and can also be obtained using internet search engines. An overview of patent information in biotechnology and a survey of relevant sources of patent information useful for retrospective searching and for current awareness is given in Berks, TIBTECH 12 (1994), 352-364.

[0264] The above disclosure generally describes the present invention. Unless otherwise stated, a term as used herein is given the definition as provided in the Oxford Dictionary of Biochemistry and Molecular Biology, Oxford University Press, 1997, revised 2000 and reprinted 2003, ISBN 0 19 850673 2. Several documents are cited throughout the text of this specification. Full bibliographic citations may be found at the end of the specification immediately preceding the claims. The contents of all cited references (including literature references, issued patents, published patent applications as cited throughout this application and manufacturer's specifications, instructions, etc) are hereby expressly incorporated by reference; however, there is no admission that any document cited is indeed prior art as to the present invention.

[0265] A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only and are not intended to limit the scope of the invention.

EXAMPLES

[0266] The examples which follow further illustrate the invention, but should not be construed to limit the scope of the invention in any way. Detailed descriptions of conventional methods, such as those employed herein can be found in the cited literature; see also "The Merck Manual of Diagnosis and Therapy" Seventeenth Ed. ed by Beers and Berkow (Merck & Co., Inc. 2003).

[0267] The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. For further elaboration of general techniques useful in the practice of this invention, the practitioner can refer to standard textbooks and reviews in cell biology and tissue culture; see also the references cited in the examples. General methods in molecular and cellular biochemistry can be found in such standard textbooks as Molecular Cloning: A Laboratory Manual, 3rd Ed. (Sambrook *et al.*, Harbor Laboratory Press 2001); Short Protocols in Molecular Biology, 4th Ed. (Ausubel *et al.* eds., John Wiley & Sons 1999); DNA Cloning, Volumes I and II (Glover ed., 1985); Oligonucleotide Synthesis (Gait ed., 1984); Nucleic Acid Hybridization (Hames and Higgins eds. 1984); Transcription And Translation (Hames and Higgins eds. 1984); Culture Of Animal Cells (Freshney and Alan, Liss, Inc., 1987); Gene Transfer Vectors for Mammalian Cells (Miller and Calos, eds.); Current Protocols in Molecular Biology and Short Protocols in Molecular Biology, 3rd Edition (Ausubel *et al.*, eds.); and Recombinant DNA Methodology (Wu, ed., Academic Press). Gene Transfer Vectors For Mammalian Cells (Miller and Calos, eds., 1987, Cold Spring Harbor Laboratory); Methods In Enzymology, Vols. 154 and 155 (Wu *et al.*, eds.); Immobilized Cells And Enzymes (IRL Press, 1986); Perbal, A Practical Guide To Molecular Cloning (1984); the treatise, Methods In Enzymology (Academic Press, Inc., N.Y.); Immunochemical Methods In Cell And Molecular Biology (Mayer and Walker, eds., Academic Press, London, 1987); Handbook Of Experimental Immunology, Volumes I-IV (Weir and Blackwell, eds., 1986). Protein Methods (Bollag *et al.*, John Wiley & Sons 1996); Non-viral Vectors for Gene Therapy (Wagner *et al.* eds., Academic Press 1999); Viral Vectors (Kaplitt & Loewy eds., Academic Press 1995); Immunology Methods Manual (Lefkovits ed., Academic Press 1997); and Cell and Tissue Culture: Laboratory Procedures in Biotechnology (Doyle & Griffiths, John Wiley & Sons 1998). Reagents,

cloning vectors and kits for genetic manipulation referred to in this disclosure are available from commercial vendors such as BioRad, Stratagene, Invitrogen, Sigma-Aldrich, and ClonTech.General techniques in cell culture and media collection are outlined in Large Scale Mammalian Cell Culture (Hu *et al.*, Curr. Opin. Biotechnol. 8 (1997), 148); Serum-free Media (Kitano, Biotechnology 17 (1991), 73); Large Scale Mammalian Cell Culture (Curr. Opin. Biotechnol. 2 (1991), 375); and Suspension Culture of Mammalian Cells (Birch *et al.*, Bioprocess Technol. 19 (1990), 251); Extracting information from cDNA arrays, Herzel *et al.*, CHAOS 11 (2001), 98-107.

[0268] The following experiments are illustrated and described with respect to antibody NI-101.11. However, the other antibodies of the NI 101 series, in particular NI 101.10 are structurally similar and thus may be expected to provide comparable results.

Supplementary Methods

Memory B cell display

[0269] Clinically carefully selected human subjects who are characterized by unusually positive clinical courses, *e.g.* absence of clinical signs of disease in the presence of risk factors, or stable courses of mild or prodromal signs with no disease development, or long-term non-progressors are recruited to provide peripheral blood lymphocytes as the starting material for the isolation of memory B cells. The strategy is based upon the concept established in infection immunology that a subject's memory B cell pool preserves the antibody specificities and possibly also antibody frequencies generated during previous antigen encounters (McHeyzer-Williams and Ahmed Curr. Opin. Immunol. 11 (1999), 172-179; Bernasconi *et al.*, Science 298 (2002), 2199-202; Traggiai *et al.*, Nat. Med. 10 (2004), 871-875). This concept was developed to explain adaptive immunity against infectious agents, as well as for the description of antibody-mediated immunity following a primary infection. According to this theory, the entire complement of antibodies against all antigens that had had induced an antibody response in the subject's history, either naturally or upon vaccination, should be fully represented within the memory B cell pool. In accordance with the present invention this theory is applied to *endogenous* antigens generated as a result of *abnormal aggregation or conformation* of an otherwise physiological relevant protein, and that, as such, is not subject to the physiological

immunologic tolerance, and, thus, can acquire antigenic properties and induce an immune response against the conformational *neo-epitopes* (*neoepitopes*).

[0270] Memory B cells are isolated with surface markers including the pan B cell marker CD22, combined with negative selection of antigen-inexperienced B cells that expressed IgM, IgD, IgE, and IgA. With this technique, approximately 10.000 to 150.000 memory B cells can be obtained from 30ml of human blood. These are immortalized, for example with Epstein Barr Virus, and cultured oligo-clonally on irradiated human fibroblast feeder layers (Zubler *et al.*, J Immunol. 134 (1985), 3662-3668; Traggiai *et al.*, Nat. Med. 10 (2004), 871-875). To improve transformation and immortalization efficacy of antibody-secreting memory B cells, CpG 2006 which mimics the activities of bacterial un-methylated CpG-dinucleotides (Hartmann and Krieg J Immunol 164(2) (2000), 944-953) can be used.

Experimental protocol:

[0271] Selection of B cells from the bulk of PBL was performed using the MACS technology and CD22 microbeads (Miltenyi, Bergisch Gladbach, Germany). PBL were labeled with MACS anti human CD22, phycoerythrin-conjugated anti human IgD and APC-conjugated antibodies anti human IgM, IgA, CD3, CD8, CD56 (Becton Dickinson, Basel, Switzerland). CD22-positive cells were isolated using LS columns and the Midi MACS device (Miltenyi) followed by selection of phycoerythrin- and APC-negative cells using a MoFlo cell sorter (Dako, Fort Collins, USA). CD22-positive, IgM-, IgD-, IgA–, and IgE-negative B cells were then incubated with Epstein Barr Virus containing supernatant obtained from B95-8 cells and CpG 2006 (Sigma, Buchs, Switzerland) at a concentration of 2.5mg/l in B cell medium (RPMI 1640 supplemented with 10% fetal calf serum (Hyclone, Perbio, Lausanne, Switzerland). 5-50 cells were seeded per well in Costar round bottom 96 well plates (Corning, Vitaris, Baar, Switzerland) in B cell medium on 30.000 irradiated human PBL prepared from voluntary donors. Memory B cell cultures were maintained at 37°C and 5% CO_2 in a humidified cell culture incubator for 2-4 weeks after which time the conditioned medium of the cultures was assayed in ELISA and on tissue arrays.

Antibody screening

[0272] Antibodies in conditioned media are screened for binding to pathological epitopes including protein aggregates and abnormal, pathologically relevant structures on tissue sections obtained from human patients with pathologically confirmed diagnoses including, but not restricted to, Alzheimer's disease, or obtained from tissue sections of transgenic mouse models of human disease, or from tissue sections obtained from animal models of human disease including aged non-human primates, or by ELISA of aggregated synthetic peptide preparations. Abnormal, pathologic structures in the sense of this invention include, but are not restricted to, ß-amyloid plaques, neurofibrillary tangles, alpha-synuclein aggregates in Lewy bodies, and protein aggregates deposited in dystrophic neurites. Human tissues are also used to exclude cross-reactivities of antibodies with normal cellular or supercellular tissue structures. Selected antibodies are further analyzed for class and light chain subclass determination. Selected pathology-relevant antibody messages from memory B cell cultures are transcribed by using RT-PCR, cloned and combined into expression vectors for recombinant production.

Experimental protocol:

[0273] Screening of B cell conditioned medium using microtiter-compatible tissue microarrays.

Array production

[0274] Paraffin-embedded human post-mortem Alzheimer's disease brain tissues were cut into rods of 1-2 mm in diameter and 10mm in length. Four rods were embedded vertically in paraffin to form a square fitting the microtiter format of 9 by 9 mm. 5 µm tissue slices were cut from this assembly with a microtome and two slices were mounted adjacent to each other onto glass slides resulting in an assembly of 2 by 4 rods that fit the 96-well microtiter format. Alternatively, tissues from APP transgenic mice were used to prepare the tissue arrays.

B cell screening

[0275] Conditioned medium from memory B cell cultures was transferred onto the tissue array slides using a multichannel pipette and incubated for 2 h at room temperature. After a washing step the binding of human antibodies to tissue sections was analyzed using Cy

3-conjugated secondary antibodies to human IgG (Jackson ImmunoResearch Europe Ltd., Suffolk, UK). Analysis of fluorescence was performed on an inverted fluorescence microscope (Leica, Heerbrugg, Switzerland).

ELISA

[0276] 96 well half area Microplates (Corning) were coated with synthetic Abeta-peptide at a standard concentration of 1 µg/ml in coating buffer (15mM Na_2CO_3, 35 mM $NaHCO_3$, pH 9.42) overnight at 4°C. Plates were washed and non-specific binding sites were blocked for 1h at RT with PBS containing 2% BSA (Sigma, Buchs, Switzerland). B cell conditioned medium was transferred from memory B cell culture plates to ELISA plates and was incubated for 2 h at room temperature. Binding of human antibodies was determined using horse radish peroxidase (HRP)-conjugated donkey anti-human IgG polyclonal antibodies (Jackson ImmunoResearch Europe Ltd., Cambridgeshire, UK) followed by measurement of HRP activity in a standard colorimetric assay.

Molecular cloning of antibodies displaying specificity of interest

[0277] Living B cells of selected memory B cell cultures are harvested using a cell sorter. mRNA is prepared and immunoglobulin heavy and light chain sequences are obtained using Ig-framework specific primers for all human variable heavy and light chain framework 1 (FR1) families as 5' primers in combination with primers specific for all human J-H segments as 3' primers (Marks *et al.*, Mol. Biol. 222 (1991)., 581-597). Alternatively, single-cell RT-PCR of single sorted cells from the memory B cell culture can be used as source of Ig heavy and light chain sequences (Babcook *et al.*, Proc. Natl. Acad. Sci. USA 93 (1996), 7843-7848; Brezinschek *et al.*, J. Immunol. 155 (1995), 190-202; Coronella *et al.*, Nucleic Acids Research 28 (2000); Owens, *et al.*, J. Immunol. 171 (2003), 2725-2733). Single cell sorting preserves the correct pairing of the immunoglobulin heavy and light chains of the antibody clones originally produced in the B cell culture.

[0278] Identification of the antibody clone with the desired specificity is performed by re-screening on microtiter compatible tissue microarray and ELISA upon recombinant expression of complete antibodies. Recombinant expression of complete IgG1 antibodies is achieved upon insertion of the variable heavy and light chain sequences "in the correct reading frame" into expression vectors that complement the variable region sequence with a sequence encoding a signal peptide at the 5-prime end and at the 3'-end with a sequence

encoding the appropriate constant domain(s). To that end the primers contained restriction sites designed to facilitate cloning of the variable heavy and light chain sequences into antibody expression vectors. Heavy chain immunoglobulin are expressed by inserting the immunoglobulin heavy chain RT-PCR product in frame into a heavy chain expression vector bearing a signal peptide and the constant domains of human immunoglobulin. Kappa light chain immunoglobulin is expressed by inserting the kappa light chain RT-PCR-product in frame into a light chain expression vector providing a signal peptide and the constant domain 1 of human kappa light chain immunoglobulin. Alternatively, lambda light chain immunoglobulin is expressed by inserting the lambda light chain RT-PCR-product in frame into a lambda light chain expression vector providing a signal peptide and the constant domain 1 of human lambda light chain immunoglobulin.

[0279] Functional recombinant monoclonal antibodies are obtained upon co-transfection into HEK 293 cells (or any other appropriate recipient cell line) of a Ig-heavy–chain expression vector and a kappa or lambda Ig-light–chain expression vector. Recombinant human monoclonal antibody is subsequently purified from the conditioned medium using a standard Protein A column purification. Recombinant human monoclonal antibody can be produced in unlimited quantities using either transiently or stably transfected cells. Cell lines producing recombinant human monoclonal antibody can be established either by using the Ig-expression vectors directly or by re-cloning of Ig-variable regions into different expression vectors. Derivatives such as F(ab), $F(ab)_2$ and scFv can also be generated from these Ig-variable regions.

Experimental protocol:

RT-PCR of bulk B cells

[0280] Living cells as identified by their forward- and sideward light scattering properties of selected memory B cell cultures were sorted in aliquots of 100-2000 cells directly in 0.2 ml PCR tubes filled with 20µl of RNAlater (Ambion, Huntingdon, UK) using a MoFlo cell sorter. mRNA was prepared using the mRNA-Direct Micro Kit (Dynal, Invitrogen, Basel, Switzerland). cDNA was prepared using the "RT for PCR" Kit (Clontech BectonDickinson, Basel, Switzerland) and PCR of immunoglobulin (Ig) heavy and light chain variable sequences was performed using the Advantage 2 PCR Kit (Clontech) using specific primers for all human variable heavy and light chain frame work 1 (FR1) families

as 5' primers in combination with primers specific for the constant domains of human Ig heavy or Ig kappa or Ig lambda light chains as 3' primers. Primers were purchased from Microsynth (Balgach, Switzerland).

[0281] A signal peptide that was used in all expression vectors was derived from the human immunoglobulin kappa light chain family 1 L5 sequence (MDMRVPAQLLGLLLLWFPGSRC; SEQ ID NO: 2) as described at V-Base and designed to provide the restriction site Xba 1 in order to facilitate the cloning of PCR amplified variable regions (ATGGACATGCGGGTGCCCGCCCAGCTGCTGGGCCTGCTGCTGCTGTGGTTCCCCGGCTCTAGATGC; SEQ ID NO: 1). Xba1 was introduced by silent mutagenesis. As a 3' restriction site used for the cloning of variable heavy chain regions the restriction site Sal1 was introduced into C1 of IgG1provided by the vector. Similarly, the restriction site BsiW1 was introduced into C1 of the kappa light chain and Xho1 was introduced into C1 of lambda light chain. Restriction digest of PCR products and ligation into to recipient vectors was performed according to standard procedures. Plasmid DNA was prepared using standard kits (Quiagen, Hombrechtikon, Switzerland). Recipient vectors contained a CMV promoter for the expression of antibody genes in mammalian cells.

Single-cell RT-PCR

[0282] For single cell RT-PCR of Ig heavy and light chain variable regions from cultured B cells a modification of the method described by Owens *et al.* was used (Owens *et al.*, 2003). Single cells were deposited directly into each tube of an array of 0.2 ml PCR tubes using the MoFlo cell sorter. Each tube was prepared to contain 10µl of RT buffer for Superscript II reverse transcriptase (Invitrogen). PCR tubes were shock frozen on dry ice and thawed immediately prior to RT-PCR. cDNA was prepared using random hexamers (Clontech) and Superscript II reverse transcriptase (Invitrogen). 1st round PCR of immunoglobulin heavy and light chain variable regions was performed using, as 5' primers, a set of primers that primed in all signal peptides conserved among Ig-variable region families. At the 3' position, single primers specific for the constant region of Ig C1 heavy chain or the Ig kappa- or Ig lambda light chain were used. 2nd round PCR was performed on PCR-products obtained during first round PCR using the primers as described for the bulk B cell RT-PCR. Cloning into recipient vectors was performed accordingly.

Alternative cellular cloning

[0283] Cloning was performed using the standard limited dilution method or by single cell deposition into 96 well culture plates using a cell sorter (MoFlo, Dako, Fort Collins, USA). For limiting dilution, cells of a memory B cell culture were harvested, passed through a 30µm nylon mesh (Falcon, Becton Dickinson, Basel, Switzerland) resuspended in medium and seeded onto 96 well plates or 384 well plates at a concentration of 0.3 cells per well.

[0284] For seeding with the cell sorter, the device was set to deposit one single cell (single 1 mode) per well directly in 96 well plates supplemented with B cell medium. The culture medium was supplemented with medium conditioned by activated T cells. Alternatively, 30.000 irradiated feeder cells were added to the medium.

Sequence analysis of immunoglobulin variable region sequences

[0285] Sequencing of cloned immunoglobulin variable region sequences was performed using primers specific for the CMV promoter present 5' of the inserted Ig variable region sequences in the expression vector. Alternatively, primers that primed in the constant domains of Ig heavy- and light chains were used. Sequences obtained were analyzed and aligned using Vector NTI software (Informax-Invitrogen). Plasmids containing sequences that encoded complete immunoglobulin variable regions in frame with the leader peptide and the constant domain were used for expression.

Expression of functional recombinant monoclonal antibodies

[0286] The antibodies can be produced in sufficient quantities by recombinant expression using technologies known in the art (Trill *et al.*, Curr. Opin. Biotechnol. 6 (1995), 553-601). Recombinant human monoclonal antibody of up to 1 mg was produced upon transient transfection of 293 HEK cells. Recombinant human monoclonal antibody of up to 100 mg was produced upon stable transduction of 293 HEK cells or the murine NSO cells using recombinant lentivirus vectors.

Small scale production of human recombinant antibody by transient transfection

[0287] Ig-heavy chain vector and Ig-light chain vectors were co-transfected into HEK 293 cells using the standard calcium phosphate co-precipitation method. Recombinant antibodies were purified from the medium conditioned by transfected HEK 293 cells using protein A column purification (GE-Healthcare, Otelfingen, Switzerland).

Large scale production of human recombinant antibody by stable transduction

[0288] Here, a lentivirus-based transfection system was employed to generate stably transduced cell lines producing human recombinant antibody (Zufferey *et al.*, J. Virol. 72 (1998), 9873-9880). HEK 293 cells were co-transduced with two distinct lentivectors one bearing an expression cassette for the Ig heavy chain, the other a cassette for the Ig light chain of a recombinant antibody. This method of transduction can be used in a broad range of mammalian cell lines such as CHO and NSO cells.

Validation in transgenic mouse models of human disease

[0289] Transgenic mice were generated as previously described (Knobloch *et al.*, Neurobiol. Aging July 28 (2006)) on a hybrid background of C57Bl/6 and DBA2. The test group was backcrossed once to C57Bl/6. Mice were kept under standard housing conditions on a reversed 12h:12h light/dark cycle and had free access to food and water. The treatment groups were balanced for age (24 months at first testing, 26 months at 2nd testing) and gender. Mice are treated with antibodies (3mg/kg body weight) by once weekly intraperitoneal injections over a time period of 2 months resulting in 8 injections per animal.

Behavioral testing in the Y-maze

[0290] The spontaneous alternation rate is assessed using a Y-shaped plastic maze, with 40 x 20 x 10 cm arm sizes. During 5 min sessions, the sequences of arm entries are recorded; alternation was defined as successive entries into the three arms, in overlapping triplet sets. The percent alternation was calculated as the ratio of actual to possible alternations. After 2 months of antibody treatment, the mice are retested in the Y-maze. The experimenters are always kept blind for both treatments and genotypes during the whole experiment.

Blood-brain barrier penetration and binding to abnormal structures in the brain

[0291] To assess whether the selected antibodies or fragments thereof can penetrate the blood-brain barrier and bind to their abnormally aggregated or conformationally altered protein targets in the brain, an effective dose of the antibody is administered systemically, intraperitoneally, intravenously, intramuscularly, subcutaneously or intranasally to a transgenic animal that is characterized by unphysiological accumulation of the aggregated or conformationally altered protein target in the brain. Binding of the antibody to the

pathology specific structures in the brain is then evaluated by immunostaining with a labeled anti-human Ig secondary antibody followed by standard immunohistochemical detection.

Experimental protocol:

[0292] PS-1/APPswe transgenic model mice for Alzheimer's disease received two peripheral injections of 150µg NI-101.11 at day 1 and day 3. The mice were sacrificed 24 h after the second injection and perfused with PBS. Brains were frozen and tissue slices were prepared from frozen tissue using a cryotome. Presence of human antibody on cryostat sections was assayed by staining with Cy3-labeled anti human IgG antibody (Jackson ImmunoResearch Europe, Suffolk, UK). Localization of amyloid plaques was performed by co-staining the cryostat sections with the murine Abeta-specific control antibody 6E10 (available from Covance, Catalog Number SIG-39320) followed by FITC-labeled anti mouse IgG antibody. Alternatively, staining with Cy3-labeled anti human IgG antibody was used alone. Analysis of fluorescence was performed on an inverted fluorescence microscope (Leica).

Reduction of brain pathology

[0293] The effects of antibody treatment on the levels of aggregated or conformationally altered protein targets in the brain is assessed by systemic treatment or targeted brain delivery of the antibody (intracranial, intrathecal or intraventricular) and an unrelated antibody control to transgenic animals with characteristic unphysiological accumulation of the aggregated or conformationally altered protein target in the brain. The treatment effects is evaluated by immunostaining or histochemical staining of the altered or aggregated protein targets and measuring the area covered by such aggregates, aggregate size and aggregate number and biochemical quantification of the concentrations of the protein targets in different brain areas.

Absence of antibody-treatment related side effects

[0294] Potential target related adverse effects of the antibody-treatment will be assessed by systemic administration or targeted brain delivery of the antibody (intracrainial, intrathecal or intraventricular) and an unrelated antibody control to transgenic animals with characteristic unphysiological accumulation of the aggregated or conformationally altered

protein target in the brain. Potential side effects will be evaluated by immunostaining or histochemical staining (*e.g.* Prussian blue for micorhemorrhages, hematoxilin-eosin, activated white blood cells) and biochemical quantification (*e.g.* cytokine levels by ELISA).

Immunofluorescence staining of living cells

[0295] HEK 293 cells were transiently transfected with a vector that expresses human wild type APP fused at the intracellular C-terminus to the yellow fluorescent protein variant Citrine. 24 hours after transfection the cells were incubated with human recombinant antibodies or control antibodies at 4°C for 30 minutes. After a washing step the cell were fixed and surface-bound antibody was detected using Cy-3-labeled secondary antibodies to human or mouse IgG (Jackson ImmunoResearch). Analysis of fluorescence was performed on a confocal microscope (Leica).

Preparation of Abeta fibrils

[0296] Abeta peptide was purchased from Bachem (Bubendorf, Switzerland). Lyophylised peptide was reconstituted in TFA and resuspended in PBS immediately prior to its use as monomeric Abeta in the assays. Abeta fibrils were prepared by incubation of monomeric Abeta1-42 peptide at a concentration of 100µg/ml in PBS at 37°C for 24h. Monomeric Abeta peptide and fibril preparations were also used as substrate to coat ELISA plates.

Western blotting

[0297] Monomeric Abeta peptide was mixed with loading dye, heat denatured and 0.2µg was loaded per lane and separated on a gradient SDS-PAGE. Blots were incubated with primary antibody for 2h. Binding of primary human monoclonal antibody or mouse control antibody 6E10 was revealed using secondary anti human or anti mouse antibodies conjugated with horse radish peroxidase (HRP). Blots were developed using SuperSignal West Femto Maximum Sensitivity Substrate (Pierce, Fisher Scientific, Wohlen, Switzerland).

Competition of tissue amyloid plaque binding

[0298] Recombinant human NI-101.11 antibody was incubated for 2 h with Abeta peptide preparations. The antibody/Abeta preparations were then used for immunohistochemical staining of brain section obtained from a patient with neuropathologically confirmed Alzheimer's disease. 5 µm cryo-sections were prepared, blocked with 4% BSA, 5% goat serum and 5% horse serum in PBS for 1h at RT and stained with NI-101.11/Abeta preparations for 1 h at room temperature. After a washing step the binding of human antibodies to tissue sections was analyzed using Cy3-conjugated secondary antibodies to human IgG (Jackson ImmunoResearch Europe Ltd). Analysis of fluorescence was performed on an inverted fluorescence microscope (Leica, Heerbrugg, Switzerland).

Example 1

Detection of Human Antibodies against Abnormal Structures Prevalent in Human Brain Diseases

[0299] Antibodies from phenotypically healthy subjects, or clinically unusually stable patients with Alzheimer's disease were tested by immunohistochemistry on brain sections obtained from patients with pathologically confirmed Alzheimer's disease. Fig. 1A demonstrates the presence of antibodies in a clinically unusually stable patient that bind to beta-amyloid plaques as was confirmed by co-staining with a known antibody against human beta-amyloid (antibody 4G8; Fig. 1B). The presence in a healthy human subject of antibodies to neurofibrillary tangles in a tissue section obtained from a patient with Alzheimer's disease is shown in Figure 2A. This result was confirmed by co-staining with a known antibody against human tau (HT7). Figure 3A reveals the presence in a healthy human subject of antibodies against dystrophic neurites in a tissue section obtained from a patient with Alzheimer's disease. Control staining with known antibody against human tau (HT7) is depicted in Figure 3 B. These results demonstrate the presence in phenotypically healthy, or clinically unusually stable patients of antibodies against identifiable pathological structures in human tissue samples with histopathologically confirmed diagnoses.

Example 2

Recombinant Human Antibodies Maintain Specificity to Abnormal Structure *in vivo* and Recognize Conformational Epitope of Disease-Associated Beta-Amyloid Protein in Brain Amyloid Plaques but not the Physiological Precursor or Non-Pathogenic Derivative Thereof

[0300] Antibodies NI-101.11, NI-101.12, NI-101.13A and NI-101.13B were obtained from clinically unusually stable Alzheimer's disease patients with a significantly reduced rate of cognitive decline. Antibody isolation and recombinant production was performed as specified in supplementary methods.

NI-101.11

[0301] Recombinant NI-101.11 was tested for binding to brain beta-amyloid plaques (Fig. 4). Brain sections obtained from a patient with neuropathologically confirmed Alzheimer's disease were stained at the indicated concentrations. Antibody binding to beta-amyloid plaques with concentrations of 50 pM suggest high affinity binding. The binding of antibody NI-101.11 to beta-amyloid plaques at a concentration of 0.5 nM cannot be competed by addition of excess amounts of linear synthetic N-terminal Abeta-derived polypeptide representing positions 1 to 16 at concentrations of up to 1 μM (Fig. 5). Furthermore, the binding of NI-101.11 to beta-amyloid plaques on brain sections at 8 nM concentration is competed by excess amounts Abeta1-42 fibrils (4 μM) but not of linear synthetic Abeta1-42 monomers at 4 μM concentration, suggesting that NI-101.11 recognizes a conformational epitope that is not present in monomeric Abeta (Fig. 6).

[0302] To further assess binding of human recombinant NI-101.11 antibody to linear, monomeric synthetic Abeta, preparations of monomeric Abeta were separated by non-denaturing PAGE. Blotted protein was probed with human recombinant NI-101.11 antibody and a control antibody against N-terminal linear Abeta sequences (6E10). While 6E10 produced prominent staining of monomeric Abeta peptide, no binding was detected for human NI-101.11 suggesting that NI-101.11 does not bind to the linear monomeric Abeta peptide but recognizes a conformational Abeta epitope. (Fig. 7)

[0303] The binding of recombinant NI-101.11 to artificial amyloid fibrils prepared from synthetic Abeta1-42 peptides and monomeric Abeta was determined by ELISA (Fig. 8). Synthetic Abeta fibrils or monomeric synthetic Abeta coated onto ELISA plates at equal coating densities were incubated with NI-101.11 at the indicated concentrations. Binding to artificial amyloid fibrils (open squares) is more than 100 times higher as compared to monomeric Abeta (filled squares). Control antibody 22C4 against the C-terminus of Abeta preferentially binds to monomeric Abeta (filled circles), and less well to fibrils (open circles). This suggests that NI-101-10 recognizes a conformational epitope which is also present on artificial amyloid fibrils prepared from synthetic Abeta peptides.

[0304] Cross-reactivity of recombinant human NI-101.11 antibody against cellular full-length APP or with any of its physiological derivatives was determined by cell binding assays (Fig. 9).

[0305] Live HEK 293 cells stably expressing human APP fused to Citrin as a marker were incubated for 30 min at 4°C, to prevent internalization, with the recombinant human NI-101.11 antibody or the control antibody 6E10 against N-terminal linear Abeta sequence. Citrin-positive signals indicate APP-expressing cells. In contrast to the control antibody (6E10) that binds to cell-surface APP in all cells expressing the fusion construct, no binding of recombinant human NI-101.11 antibody to full-length APP is detected. These data demonstrate absent cross-reactivity of NI-101.11 to physiological, cellular APP.

[0306] The lack of binding of NI-101.11 to monomeric Abeta was further demonstrated by size exclusion chromatography: No binding of NI-101.11 or an unrelated control antibody was observed to monomeric FITC-labeled Abeta1-42 (Fig. 10A, 10B). In contrast, antibody 22C4 directed against a linear epitope present in the C-terminus of Abeta co-eluted with FITC-Abeta1-42 monomers (Fig. 10 C).

[0307] In a competition ELISA, binding of 6E10, an antibody directed against a linear epitope at the N-terminus of Abeta, could be completely blocked upon pre-incubation with excess concentrations of monomeric Abeta1-16, Abeta1-28 and Abeta1-40 peptides. In contrast, pre-incubation with excess concentrations of linear Abeta peptides did not abolish NI-101.11 binding, suggesting that NI-101.11 requires a conformational epitope (Fig. 11).

NI-101.13A and 13B

[0308] Recombinant human antibodies NI-101.13A and 13B were tested for binding to brain sections obtained from an APP transgenic mouse model of Alzheimer's disease (Tg2576). NI-101.13A and NI-101.13B produced prominent staining of beta-amyloid plaques at 10 nM concentration (Fig. 12). The binding of recombinant NI-101.13A and NI-101.13B to artificial amyloid fibrils prepared from synthetic Abeta1-42 peptides and monomeric Abeta was determined by ELISA. Synthetic Abeta fibrils or monomeric synthetic Abeta coated onto ELISA plates at equal coating densities were incubated with NI-101.13A and NI-101.13B at the indicated concentrations. Preferential binding to artificial amyloid fibrils as compared to monomeric Abeta was observed for both antibodies tested. (Fig. 13)

NI-101.12

[0309] The binding of recombinant NI-101.12 to synthetic Abeta1-42 peptide was confirmed by ELISA (Fig. 14A). NI-101.12 binding at 133nM concentration was competed by excess of Abeta1-42 peptide (Fig 14 B).

Example 3

Recombinant Human Antibody against Brain Beta-Amyloid Crosses the Blood Brain Barrier in a Transgenic Mouse Model of Alzheimer's Disease, and Binds to Brain Beta-Amyloid Plaques *In vivo*

[0310] To determine whether recombinant human NI-101.11 antibody crosses the blood brain barrier and binds to brain beta-amyloid plaques *in vivo* transgenic PS-1/APPswe Alzheimer's disease model mice received two peripheral injections of 150μg NI-101.11 at day 1 and day 3. Mice were sacrificed 24 h after the second injection and perfused with PBS. Brains were harvested and brain sections were stained with FITC-labeled antibodies against human IgG or with the mouse monoclonal Abeta antibody 6E10 followed by a FITC-labeled antibody against mouse IgG to confirm the presence of brain beta-amyloid plaques. Intense staining of amyloid plaques with anti-human IgG indicated that the

recombinant human NI-101.11 antibody can cross the blood-brain barrier of transgenic mice and bind to brain beta-amyloid plaques in living animals (Fig. 15).

Example 4

Recombinant Human Antibody against Beta-Amyloid Improves Abnormal Cognitive Behavior and Confers Reduction of Beta-Amyloid Plaque Load, Astrogliosis and Microgliosis in a Transgenic Mouse Model of Alzheimer's Disease without Increasing the Frequency of Microhemorrhages

[0311] 24 months old arcAbeta mice and age-matched wild type littermates were treated weekly i.p. with 3 mg/kg recombinant human NI-101.11 antibody or an isotype-matched human control antibody for 2 months. To assess the treatment effect on abnormal behavior in the transgenic mice, Y-maze behavioral testing was performed before and after completion of the treatment. The spontaneous rate of alternation was assessed using a Y-shaped plastic maze, with 40 x 20 x 10 cm arm sizes. During 5 min sessions, the sequences of arm entries were recorded; alternation was defined as successive entries into the three arms, in overlapping triplet sets. The percent alternation was calculated as the ratio of actual to possible alternations (defined as the total number of arm entries – 2) multiplied by 100%. The Y-maze performance of untreated arcAbeta mice and wildtype littermate controls was compared using an unpaired t-test. The nonparametric Kruskal-Wallis test was used to compare the improvement after treatment in all 4 groups. The nonparametric Mann-Whitney U test was chosen for pair-wise comparison of the different groups. Zero-performers (i.e. mice that did not leave the arm they were placed in) were excluded from the analysis.

[0312] As was observerd in previous studies, untreated 24-months old arcAbeta mice were significantly impaired compared to their wildtype littermates (Fig. 16A , before treatment; unpaired t-test, p=0.0007).

[0313] NI-101.11 treated arcAbeta mice showed clearly enhanced alteration levels, comparable to NI-101.11 treated wildtype control mice after the 2 months treatment. Analysis of the improvement (i.e. performance after treatment minus performance before treatment) showed a significant difference between the four groups (Fig. 16 B, Kruskal-Wallis test; p=0.03). A pair-wise post-hoc analysis between all groups showed that NI-101.11 treated arcAbeta mice improved their cognitive performance significantly more than wildtype mice (Mann-Whitney U; p=0.05 NI-101.11 tg vs. NI-101.11 wt; p=0.008 NI-101.11 tg vs. control wt). This group of mice also showed a strong trend towards improved performance compared to the control antibody treated transgenic littermates (Mann-Whitney-U; p=0.08 NI-101.11 tg vs. control tg). All mice showed a ~10% improvement in performance in the re-testing, which was likely due to the familiar environment of the task.

[0314] The effects of chronic, 2 months NI-101.11 treatment on amyloid burden, astrogliosis and microgliosis were analyzed by quantitative histochemical and immunohistochemical analysis. To that end, mice were anesthetized after completion of the behavioral testing and perfused transcardially with PBS. One brain hemisphere was fixed in 4% paraformaldehyde and embedded in paraffin. 5 µm sagittal sections were cut with a Leica RM 2135 microtome (Bannockburn, Illinois). Beta-amyloid plaque load in cortex and hippocampus was quantified on brain sections stained with Thioflavin S and Congo Red according to standard protocol. For immunohistochemistry, slices were dewaxed, blocked with 4% BSA, 5% goat serum and 5% horse serum in PBS for 1h at RT. Antibodies were incubated overnight at 4°C using the following dilutions: anti GFAP (Advanced Immunochemicals) 1: 500, anti IBA1 (WAKO) 1:500. 2nd fluorophore coupled antibodies were incubated at RT for 2h. 2-3 sections per mouse brain spaced 75 µm apart were used for each staining. 2 images per section were taken at 10x magnification for cortex analysis (parietal and frontal region). The entire hippocampus area (5x magnification cropped to ROI) was taken for the hippocampus analysis. Automated image analysis was done with the software ImageJ.

[0315] Double staining of brain sections from immunized arcAbeta mice with 6E10 and anti-human IgG revealed binding of NI-101.11 to Abeta deposits (Fig. 17, left panel), indicating that NI-101.11 can cross the blood brain barrier and bind to brain beta-amyloid plaques. No such binding of human antibody to Abeta deposits was seen in control antibody treated arcAbeta mice (Fig. 17 right panel).

[0316] [0317] Chronic treatment with with 3mg/kg of NI-101.11 resulted in a significant reduction of amyloid plaque load as was revealed by Thioflavin S and Congo Red staining. This reduction reached levels of greater than 50% in cortex and hippocampus compared to control antibody-treated arcAbeta mice (Fig. 18 A, B). In addition to the plaque area (Fig 18 C), significant reductions were also observed for the number of plaques (Fig. 18 D) and the average plaque size (Fig. 18 E).

[0317] To test whether chronic treatment with NI-101.11 affects the neuroinflammatory response in arcAbeta mice, reactive astrocytes and microglia were quantified after immunohistological staining. A reduction in the number of reactive astrocytes (anti GFAP-staining) was observed in the cortex of NI-101.11 treated arcAbeta mice compared to control antibody treated animals (Fig. 19A; Mann-Whitney-U; p=0.047). No change was detected in the hippocampus. Staining with an antibody against a marker of microglia and macrophages (anti-Iba1) also revealed a statistical trend towards reduced inflammation (Fig. 19 B; Mann-Whitney-U; p=0.075 for both cortex and hippocampus). The decrease in astrocytosis and microgliosis is in line with the reduced beta-amyloid load observed after NI-101.11 treatment

[0318] Passive immunotherapy with certain monoclonal antibodies directed against Abeta can be associated with increased frequency of microhemorrhages in the brain (Pfeifer et al., Science 298 (2002), 1379; Wilcock et al., J Neuroinflammation 1 (2004), 24). To assess the effects of chronic therapy with NI-101.11, Perl's Prussian blue staining was performed on brain sections from arcAbeta and wild-type mice after chronic NI-101.11 treatment. This staining reveals the presence of hemosiderin, a breakdown product of hemoglobin, and marker of previous microhemorrages (Fig. 20). In aged arcAbeta mice treated with a control antibody, the frequency of Prussian blue positive profiles was significantly elevated compared to wild-type littermates (Mann-Whitney-U; p=0.001). Treatment with the NI-101.11 did not lead to an increase the number of microhemorraghes when compared to control-antibody treated arcAbeta mice (Mann-Whitney-U; p=0.347) indicating that the beneficial therapeutic effects of NI-101.11 treatment occurred in the absence of this frequently observed side effect of passive Abeta immunotherapy.

Example 5

Recombinant Human Antibody against Brain Beta-Amyloid Inhibits the Formation of Synthetic Abeta Fibrils *In vitro*

[0319] The effect of recombinant human NI-101.11 antibody on the formation of Abeta-fibrils was assayed by measuring Thioflavin S bound to aggregated Abeta by fluorescence analysis. Monomeric Abeta solutions were incubated at 37°C for 24 h in the presence of absence of increasing concentration of NI-101.11. The formation of synthetic Abeta fibrils *in vitro* was inhibited by recombinant human NI-101.11 in a concentration dependent manner (Fig. 21).

Example 6

NI-101.11 Effects on Ex-vivo Phagocytosis of Abeta Fibrils by BV-2 Microglial-derived Cells

[0320] The effects of NI-101.11 on Fcgamma-receptor mediated phagocytosis of Abeta fibrils were studied in the BV-2 microglial-derived cell line. BV-2 cells were maintained in DMEM supplemented with 5% FBS, , Pen/Step and glutamine. Cells were trypsinized and 120'000 BV-2 cells/well were seeded in flat bottom 24-well plates. After 12 h, the medium was replaced with 400 ul DMEM/F12/well supplemented with 20mM HEPES (pH 7.3), 1% BSA, 10 µg/ml Pen/Step . 100 µg/ml Fucoidan, an inhibitor of the scavenger receptor, was added 30 min prior to experiment. 50 µM FITC-labeled Abeta fibrils were pre-incubated with the indicated concentrations of antibodies for 30 min at 37°C, washed twice followed by centrifugation for 5 min at 14'000 x g. This suspension was added to the tissue culture plates. After 30 min BV-2 cells were washed twice with HBSS to remove unassociated fibrillar Abeta.

[0321] Cells were treated with 250 µg/ml trypsin/EDTA for 20 min at 4°C and washed twice by centrifugation at 500 x g for 5 min at 4°C. Cells were fixed for 20 min in FACS-Fix (PBS, 2%FA, 2% Glucose, 5 mM NaN) and washed twice with FACS wash (PBS, 5 µM EDTA, 0.2% BSA). Fluorescence (FL-1) of 10'000 cells was determined by FACS analysis (based on Webster SD et al, JI 2001).

[0322] Fcgamma receptor-dependent phagocytosis of FITC-labeled Abeta1-42 fibrils was measured upon inhibition of the scavenger receptor system. Comparative analysis of human NI-101.11 and a commercially available antibody directed to a linear epitope at the N-terminus of the Abeta peptide (6E10) demonstrated dose-dependent induction of phagocytosis of Abeta fibrils. The uptake of fibrils mediated by NI-101.11 is up to 3 fold higher than that observed for the 6E10 antibody (Fig. 22). These data indicate that NI-101.11 triggers potent dose-dependent Fcgamma receptor-mediated phagocytosis of Abeta fibrils by microglial cells.

Conclusion

[0323] As demonstrated in the above experiments performed in accordance with the present invention it was surprisingly possible to detect protective and therapeutically active antibodies and antibody producing B-cells in phenotypically healthy, asymptomatic human subjects, as well as in patients with unusually stable clinical disease courses despite a diagnosis of cognitive impairment or Alzheimer's disease. More specifically, a new class of human antibodies could be detected and isolated, which discriminate the physiologically functional form of an antigen, thereby minimizing the risk of autoimmunogenic side effects hitherto being a problem in immunotherapy. Thus, antibodies and equivalent binding molecules are provided that specifically recognize a variant of the antigen in a pathophysiologically relevant structure, which the antibody is supposed to bind in order to diminish its toxicity or to reduce its concentration or to promote its degradation, by means of, for example, making the pathogen for FcR-expressing macrophages or microglia cells visible and therefore to render it innocuous. As further demonstrated in the examples such antibodies are therapeutically effective and are capable of both suspending as well as preventing deleterious effects of abnormal pathological proteins and aggregates thereof without increasing the frequency of brain microhemorrhages.

WHAT IS CLAIMED IS:

1. A method of isolating a disorder-associated protein specific binding molecule comprising:
   (a) subjecting a sample obtained from a patient who is symptom-free but affected with or at risk of developing a disorder, or a patient with an unusually stable disease course to a specimen of pathologically altered cells or tissue of predetermined clinical characteristics; and
   (b) identifying and optionally isolating a binding molecule which binds to said specimen but not to corresponding cells or tissues of a healthy subject.

2. The method of claim 1, wherein said sample comprises a body fluid or a cell sample.

3. The method of claim 2, wherein said body fluid is a cerebrospinal fluid, plasma or 5 urine.

4. The method of any one of claims 1 to 3, wherein said binding molecule is an antibody.

5. The method of any one of claims 1 to 4, wherein said sample comprises or is derived from B-cells or memory B-cells.

6. The method of any one of claims 1 to 5, wherein said patient and subject, respectively, is a human.

7. The method of any one of claims 1 to 6, wherein said patient has been determined to be affected with a not yet manifested disorder or at risk to develop the disorder by the presence or absence of a surrogate marker.

8. The method of claim 7, wherein said surrogate marker is selected from the group consisting of old age, brain amyloid load, ApoE genotype, APP genotype, PS1 genotype, levels in body fluids of Abeta peptide, isoprostanes, Tau, and phospho-Tau.

9. The method of any one of claims 1 to 8, wherein said disorder is selected from the group consisting of neurological disorders, autoimmune disorders and tumors.

10. The method of any one of claims 1 to 9, wherein said disorder is Alzheimer's disease.

11. The method of claim 10, wherein the sample is obtained from a patient fulfilling the following criteria:

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6548

(i) being 65 years of age or older;
(ii) having full cognitive capacity and good health; and
(iii) having no clinical signs of dementia; or
(iv) having unusually slow rates of progression of disease despite the presence of an established clinical diagnosis of probable Alzheimer's disease.

12. The method of any one of claims 1 to 11 further comprising the steps of:
(i) purifying B cells or B memory cells from a sample which has been identified to contain binding molecules, *e.g.* antibodies which bind to said specimen but not to corresponding cells or tissue of a healthy subject;
(ii) obtaining the immunoglobulin gene repertoire for said antibodies from said B cells or B memory cells; and
(iii) using said repertoire to express said antibodies.

13. The method of claim 12, wherein step (ii) comprises the steps of
(iv) obtaining mRNA from said B cells or memory B cells;
(v) obtaining cDNA from the mRNA of step (iv); and
(vi) using a primer extension reaction to amplify from said cDNA the fragments corresponding to the heavy chains (HC) and the kappa light chains (LC) of said antibodies.

14. A binding molecule obtainable by the method of any one of claims 1 to 13, which is capable of selectively recognizing a neoepitope of a disorder-associated protein.

15. The binding molecule of claim 14, which does not substantially recognize said protein in its non-disorder-associated form.

16. The binding molecule of claim 14 or 15, which is an antibody or an antigen binding fragment thereof.

17. The antibody of claim 16, which is a human antibody.

18. The binding molecule of any one of claims 14 to 17, wherein said disorder is a neurological disorder.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6549

19. The binding molecule of any one of claims 14 to 18, wherein said disorder is a disorder of the brain.

20. The binding molecule of any one of claims 14 to 19, which is capable of crossing the blood brain barrier at the site of the pathological event.

21. The binding molecule of any one of claims 14 to 20, capable of binding beta-amyloid plaques, cerebrovascular amyloid, diffuse Abeta deposits, neurofibrillary tangles, hyperphosphorylated tau, alpha-synuclein positive Lewy-bodies or protein aggregates associated with dystrophic neurites.

22. The binding molecule of any of claims 14 to 21, which is capable of substantially restoring normal behavior.

23. The binding molecule of any one claims 14 to 22, which is selected from the group consisting of a single chain Fv fragment (scFv), a F(ab') fragment, a F(ab) fragment, and an F(ab')2 fragment.

24. The binding molecule of any one claims 14 to 23, which is an antibody or binding fragment thereof comprising in its variable region at least one complementarity determining region (CDR) as depicted in Table 4.

25. The antibody or binding fragment of claim 24 comprising an amino acid sequence of the V_H and/or V_L region as depicted in Tables 2 and 3.

26. An antigen which is recognized by the binding molecule of any one of claims 14 to 25.

27. The antigen of claim 26, which is at least part of a disorder-associated protein as defined in any one of the preceding claims.

28. A polynucleotide encoding the binding molecule of any one of claims 14 to 25 or the antigen of claim 26 or 27.

29. The polynucleotide of claim 28 encoding at least the variable region of an immunoglobulin chain of the antibody or binding fragment of claim 24 or 25.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6550

30. A vector comprising the polynucleotide of claim 28 or the polynucleotide of claim 29, optionally in combination with a polynucleotide of claim 29 that encodes the variable region of the other immunoglobulin chain of said antibody.

31. A host cell comprising a polynucleotide of claim 28 or 29 or a vector of claim 30.

32. A method for preparing a disorder-associated protein specific binding molecule, an antibody or a binding fragment or immunoglobulin chain(s) thereof, said method comprising

(a) culturing the cell of claim 31; and

(b) isolating said binding molecule, antibody or binding fragment or immunoglobulin chain(s) thereof from the culture.

33. A binding molecule, an antibody, an immunoglobulin chain or a binding fragment thereof encoded by a polynucleotide of claim 28 or 29 or obtainable by the method of claim 32.

34. The binding molecule, antibody or binding fragment of any one of claims 14 to 25 or 33, which is detectably labeled.

35. The binding molecule, antibody or binding fragment of claim 34, wherein the detectable label is selected from the group consisting of an enzyme, a radioisotope, a fluorophore and a heavy metal.

36. The binding molecule, antibody or binding fragment of any one of claims 14 to 25 or 33 to 35, which is attached to a drug.

37. A composition comprising the binding molecule, antibody or binding fragment of any one of claims 14 to 25 or 33 to 36, the antigen of claim 26 or 27, the polynucleotide of claim 28 or 29, the vector of claim 30 or the cell of claim 31.

38. The composition of claim 37, which is a pharmaceutical composition and further comprises a pharmaceutically acceptable carrier.

39. The pharmaceutical composition of claim 38 further comprising an additional agent useful for treating Alzheimer's disease, selected from the group consisting of small organic molecules, anti-Abeta antibodies, and combinations thereof.

40. The composition of claim 37, which is a diagnostic composition and further comprises reagents conventionally used in immuno or nucleic acid based diagnostic methods.

41. Use of the binding molecule, antibody or binding fragment of any one of claims 14 to 25 or 33 to 36 or of an antibody having substantially the same binding specificities of any one thereof, the antigen of claim 26 or 27, the polynucleotide of claim 28 or 29, the vector of claim 30 or the cell of claim 31 for the preparation of a pharmaceutical or diagnostic composition for treating or preventing the progression of Alzheimer's disease; for the amelioration of symptoms associated with Alzheimer's disease; for diagnosing or screening a subject for the presence of Alzheimer's disease or for determining a subject's risk for developing Alzheimer's disease.

42. The use of claim 41, wherein said pharmaceutical composition is administered intravenously, intramuscularly, subcutaneously, intraperitoneally, intranasally, parenterally or as an aerosol.

43. A method of treating a neurological disorder characterized by abnormal accumulation and/or deposition of a protein in the central nervous system, which method comprises administering to a subject in need thereof a therapeutically effective amount of the binding molecule of any one of claims 14 to 25 or 33 to 36, the antigen of claim 26 or 27, the polynucleotide of claim 28 or 29, the vector of claim 30 or the cell of claim 31.

44. The method of claim 43, wherein said disorder is selected from the group consisting of Alzheimer's disease, Down's syndrome, mild cognitive impairment, cerebral amyloid angiopathy, vascular dementia, multi-infarct dementia, Parkinson's disease, Huntington's disease, Creutzfeldt-Jakob disease, cystic fibrosis, or Gaucher's disease.

45. The method of claim 43 or 44, wherein administration is performed intravenously, intramuscularly, subcutaneously, intraperitoneally, intranasally, parenterally or as an aerosol.

46. A method of diagnosing and/or treating a disorder related to Alzheimer's disease and Abeta deposition, respectively, comprising administering to a subject a therapeutically effective amount of a ligand Abeta-binding molecule comprising at least one CDR of the antibody or binding fragment of claim 24 or 25 or a corresponding anti-idiotypic antibody.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6552

47. A kit for the diagnosis of a disorder which is accompanied with the presence of a disorder-associated protein as defined in any one of the peptides claimed, said kit comprising the binding molecule, antibody or binding fragment of any one of claims 14 to 25 or 33 to 36, the antigen of claim 26 or 27, the polynucleotide of claim 28 or 29, the vector of claim 30 or the cell of claim 31, optionally with reagents and/or instructions for use.

48. Use of a binding molecule, an antibody or binding fragment thereof of any one of claims 14 to 25 or 33 to 36 for *in vivo* detection of or targeting a therapeutic and/or diagnostic agent to a disorder-associated protein in the brain, detecting, suppressing formation of or reducing pathological protein aggregates or conformations in a subject, for improving cognition or slowing or reversing cognitive decline associated with diseases, or for extra-corporal extraction of pathological compounds or their precursors from body fluids.

49. An isolated antibody or antigen-binding fragment thereof which specifically binds to the same neoepitope of a disorder-associated protein as a reference antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, and NI-101.13B.

50. An isolated antibody or antigen-binding fragment thereof, wherein the antibody competitively inhibits a reference antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, and NI-101.13B from binding to the neoepitope of a disorder-associated.

51. An isolated antibody or antigen-binding fragment thereof wherein the antibody comprises an antigen binding domain identical to that of an antibody selected from the group consisting of NI-101.10, NI-101.11, NI-101.12, NI-101.13, NI-101.12F6A, NI-101.13A, and NI-101.13B.



FIG. 1

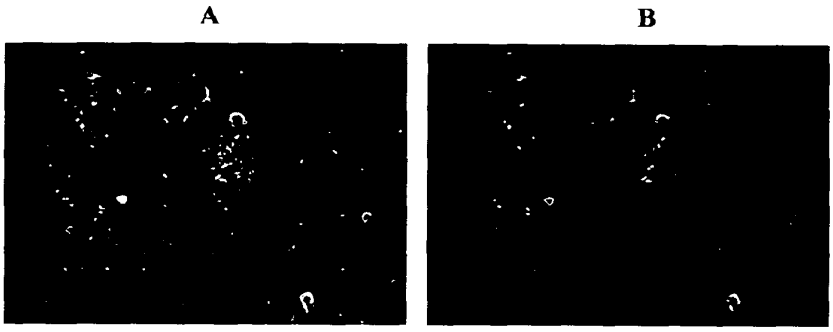


FIG. 2

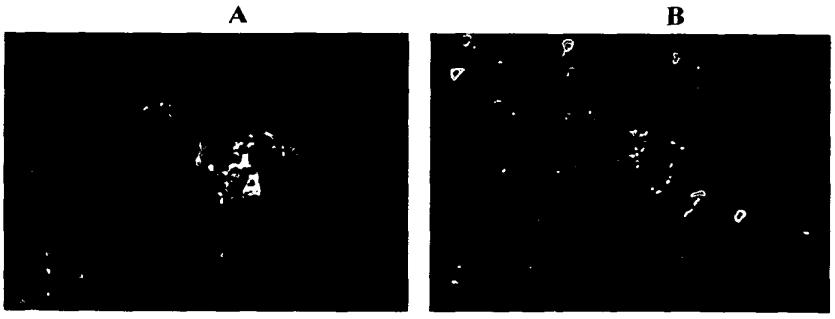


FIG. 3

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6554

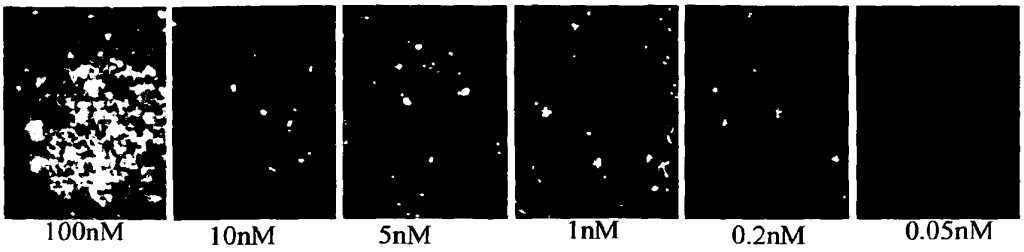


FIG. 4

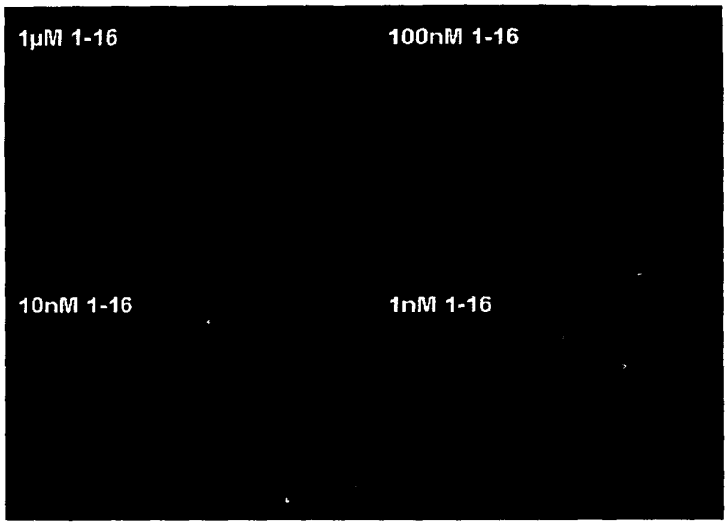


FIG. 5

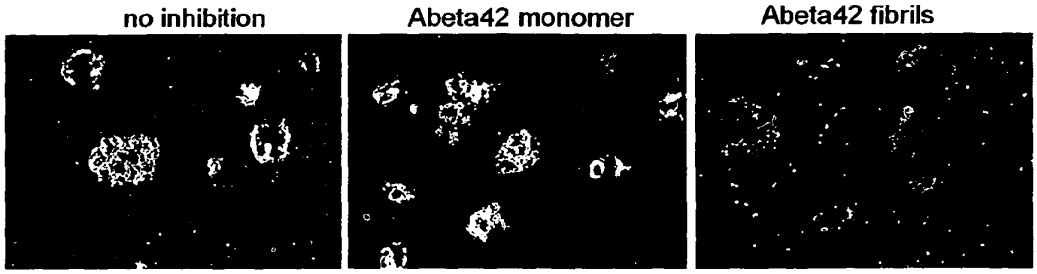


FIG. 6

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6555



FIG. 7

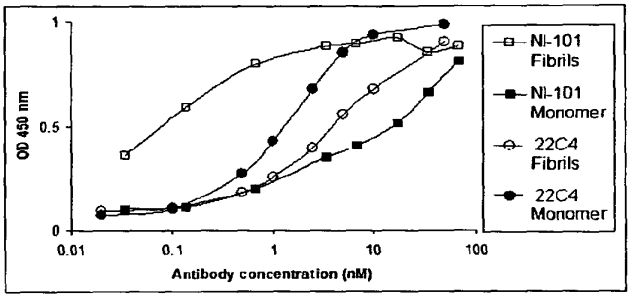


FIG. 8

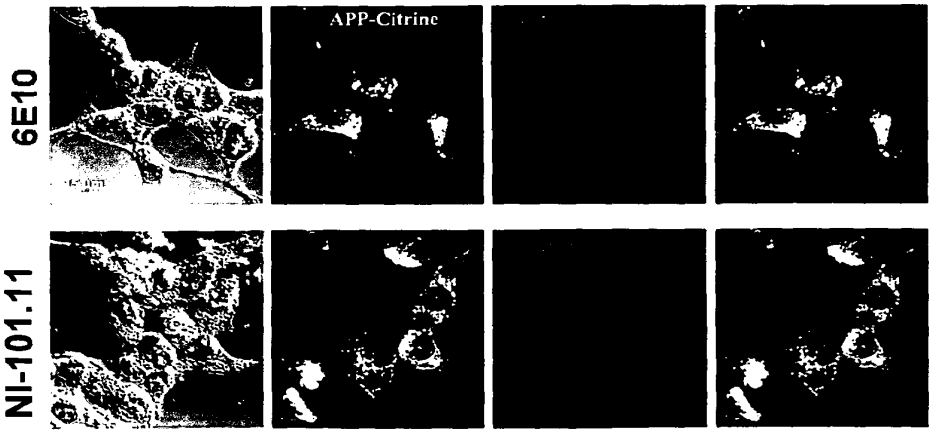


FIG. 9

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6556

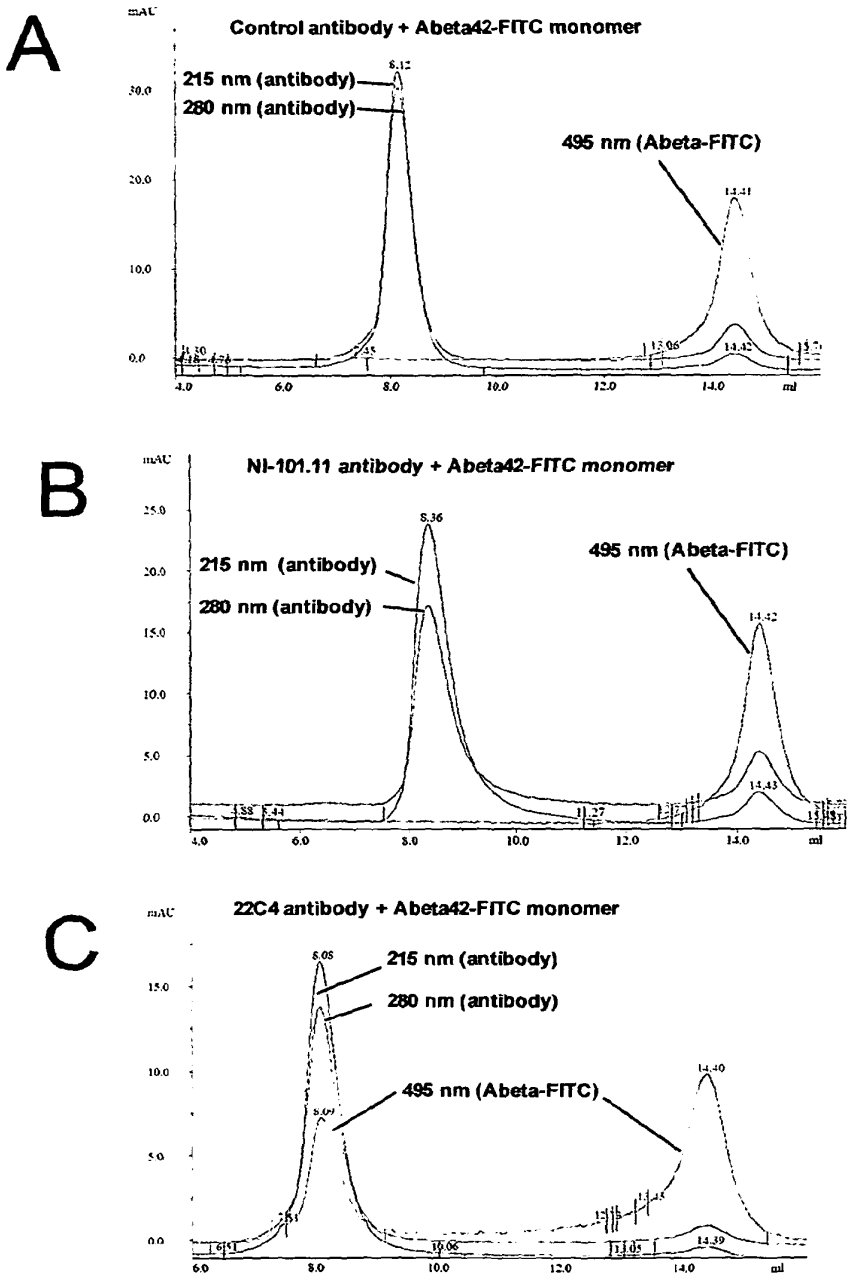


FIG. 10A-C

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6557

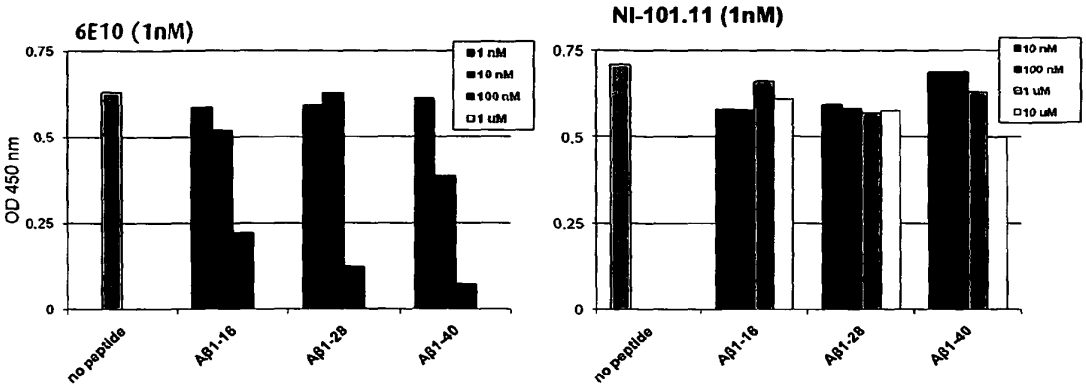


FIG. 11

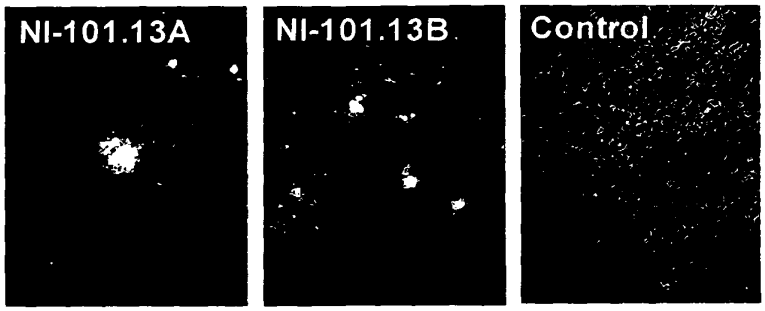


FIG. 12

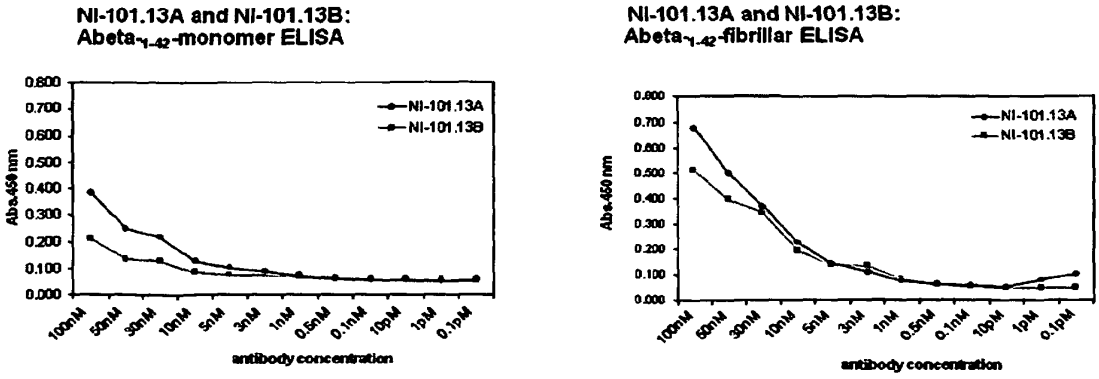


FIG. 13

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6558

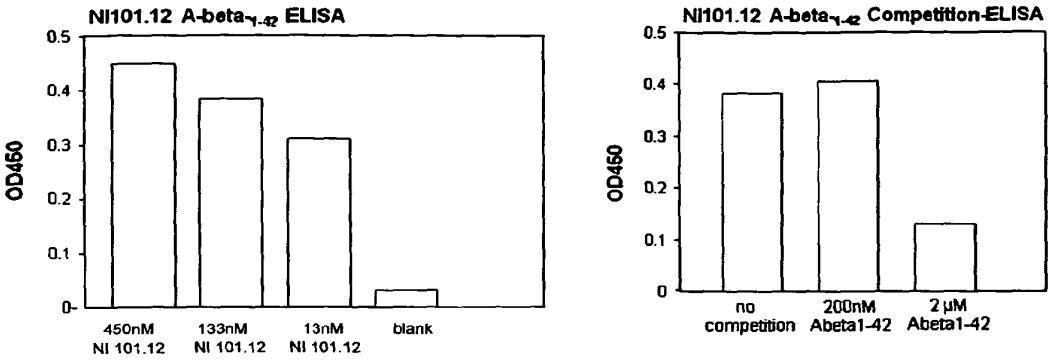


FIG. 14A-B

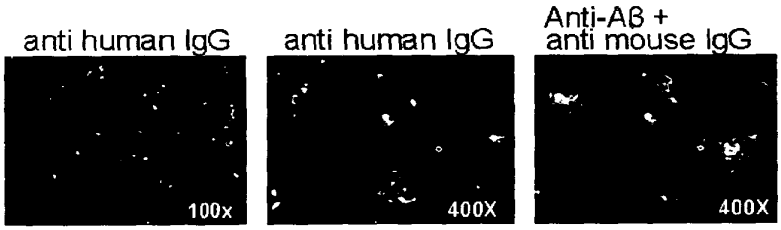


FIG. 15

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6559

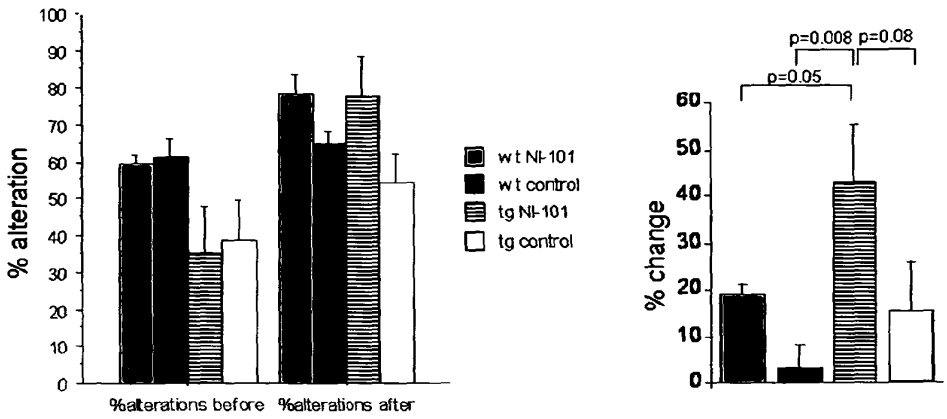


FIG. 16A-B

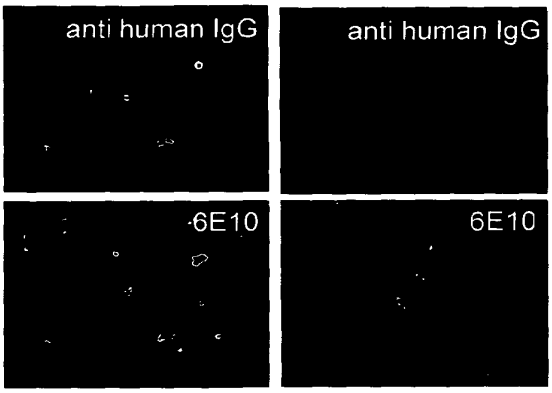


FIG. 17

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6560

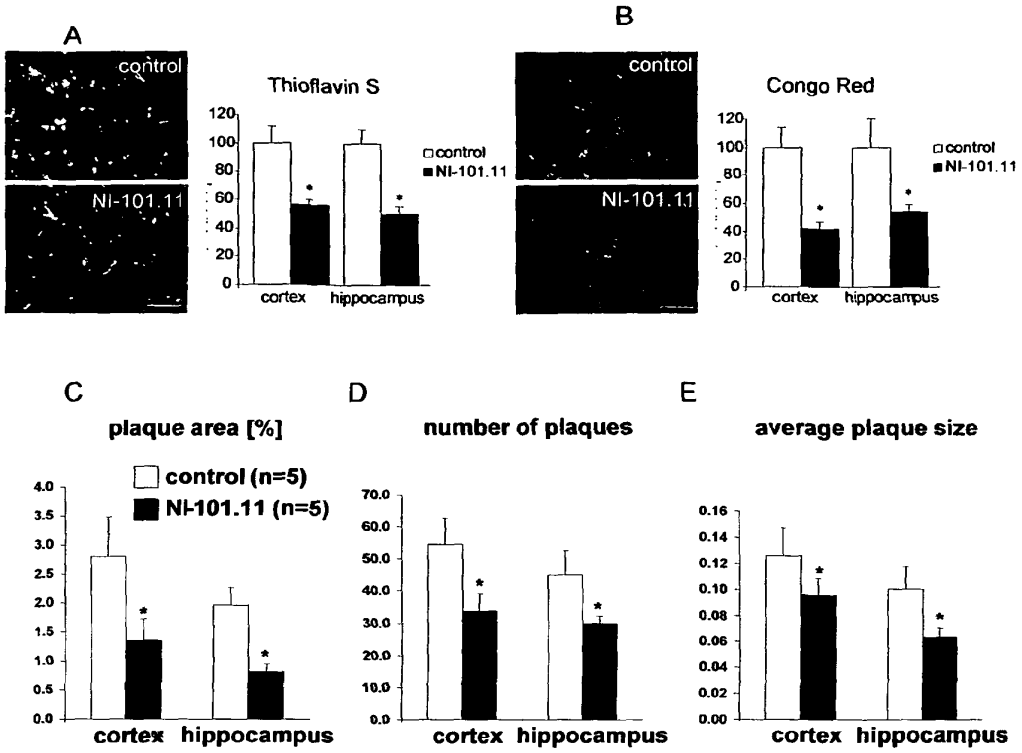


FIG. 18A-E

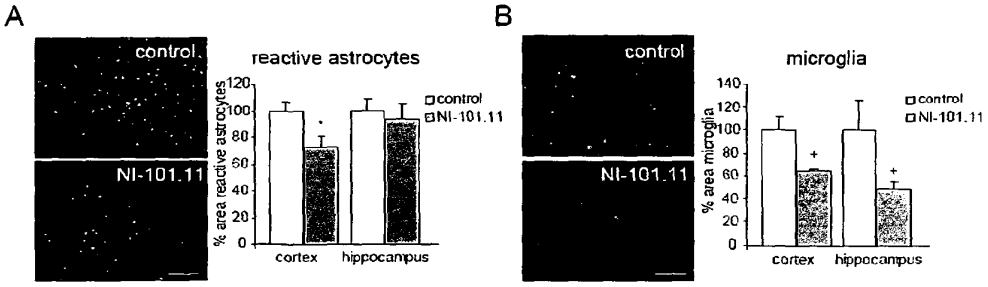


FIG. 19A-B

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6561

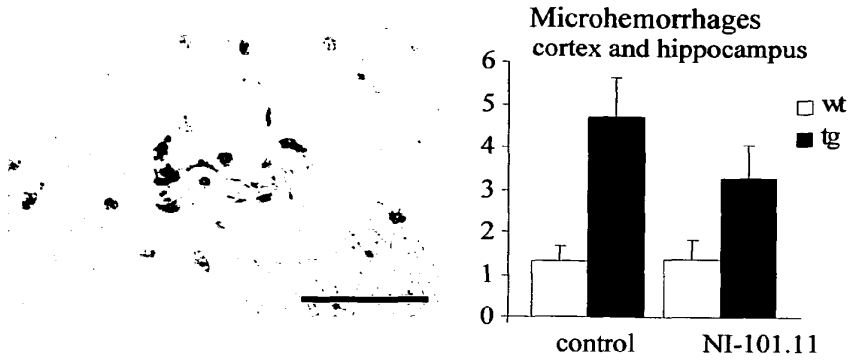


FIG. 20

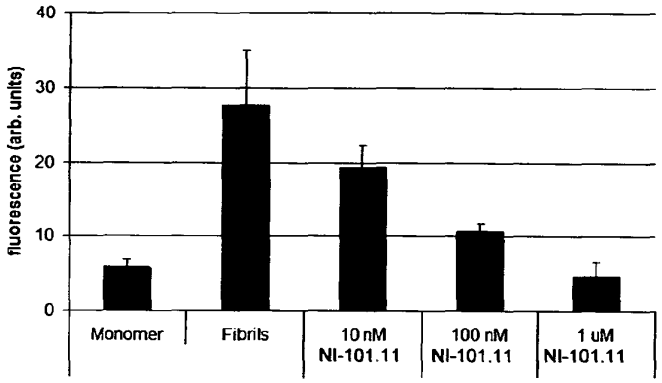


FIG. 21

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6562

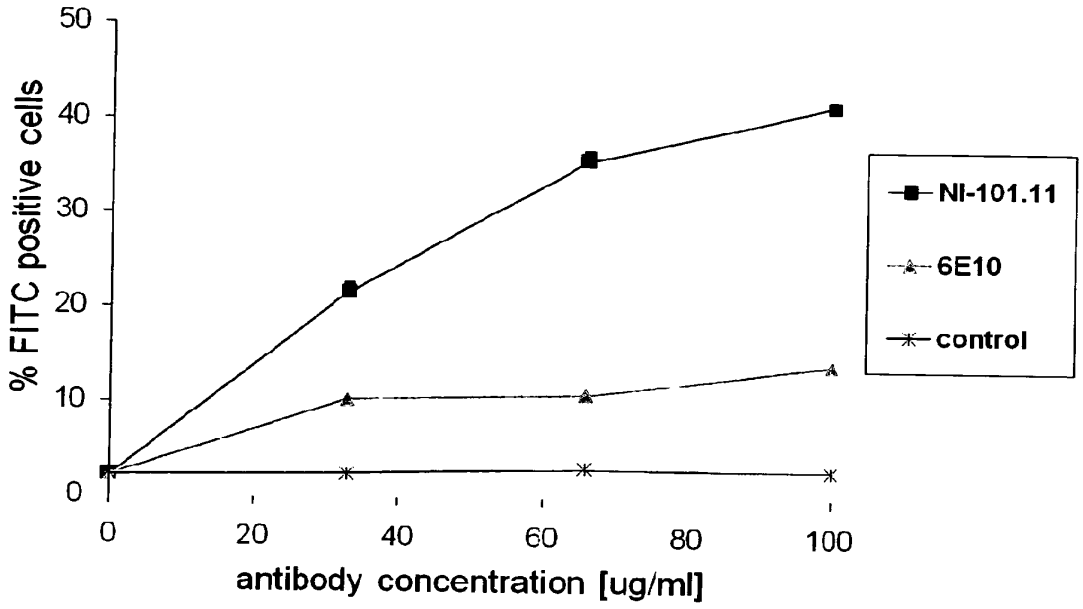


FIG. 22

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6563

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/000053

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18 A61K39/395 A61P25/28

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61K A61P

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	SIMPSON J ET AL: "Autoantibodies to Alzheimer and normal brain structures from virus-transformed lymphocytes." JOURNAL OF NEUROIMMUNOLOGY NOV 1986, vol. 13, no. 1, November 1986 (1986-11), pages 1-8, XP002430383 ISSN: 0165-5728 the whole document	1-6,9, 10, 14-19,21
Y		7,8, 11-13, 20,22-51
Y	WO 2006/103116 A (BIOTHERAPIX MOLECULAR MEDICINE [ES]; TORAN GARCIA JOSE LUIS [ES]; LAIN) 5 October 2006 (2006-10-05) cited in the application the whole document	7,8, 11-13, 20,22-51

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents :

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

Date of the actual completion of the international search	Date of mailing of the international search report
27 March 2008	09/04/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Pérez-Mato, Isabel

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/000053

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GEYLIS ET AL: "Immunotherapy of Alzheimer's disease (AD): From murine models to anti-amyloid beta (Abeta) human monoclonal antibodies" AUTOIMMUNITY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 5, no. 1, January 2006 (2006-01), pages 33-39, XP005203566 ISSN: 1568-9972 page 34, column 2, line 11 - page 35, column 2, line 12	24,25
Y	EP 1 172 378 A (DODEL RICHARD DR [DE]; YANSHENG DR DU [US]) 16 January 2002 (2002-01-16) page 2, paragraph 13 - page 3, paragraph 26	24-51
Y	WEKSLER M E ET AL: "Patients with Alzheimer disease have lower levels of serum anti-amyloid peptide antibodies than healthy elderly individuals" EXPERIMENTAL GERONTOLOGY, ELSEVIER SCIENCE, OXFORD, GB, vol. 37, no. 7, July 2002 (2002-07), pages 943-948, XP002903206 ISSN: 0531-5565 page 947, column 1, line 20 - column 2, line 31	24-51
Y	DU Y ET AL: "Human anti-beta-amyloid antibodies block beta-amyloid fibril formation and prevent beta-amyloid-induced neurotoxicity" BRAIN, OXFORD UNIVERSITY PRESS, OXFORD, GB, vol. 126, no. 9, September 2003 (2003-09), pages 1935-1939, XP002360367 ISSN: 0006-8950 the whole document	24-51
Y	GEYLIS V ET AL: "Human monoclonal antibodies against amyloid-beta from healthy adults" NEUROBIOLOGY OF AGING, TARRYTOWN, NY, US, vol. 26, no. 5, May 2005 (2005-05), pages 597-606, XP004748495 ISSN: 0197-4580 page 598, column 1, line 30 - page 604, column 1, line 10	24-51

-/--

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2008/000053

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	HYMAN B T ET AL: "Autoantibodies to Amyloid-beta and Alzheimer's Disease" ANNALS OF NEUROLOGY, BOSTON, US, vol. 49, no. 6, 2001, pages 808-810, XP001030614 ISSN: 0364-5134 the whole document	24-51
A	US 2006/235207 A1 (TSUCHIYA MASAYUKI [JP] ET AL) 19 October 2006 (2006-10-19) page 1, paragraph 4 - page 4, paragraph 66	
A	WO 2005/018424 A (RES FOUND MENTAL HYGIENE [US]; MILLER DAVID L [US]; MEHTA PANKAJ [US];) 3 March 2005 (2005-03-03) page 1, paragraph 2 - page 9, paragraph 29	
A	SIMPSON J ET AL: "Antibodies to normal and Alzheimer human brain structures from non-immunised mice of various ages." FEBS LETTERS 8 JUN 1987, vol. 217, no. 1, 8 June 1987 (1987-06-08), pages 62-64, XP002430384 ISSN: 0014-5793 the whole document	

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/EP2008/000053

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

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

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claims 43-46 are directed to a method of treatment and a diagnostic method of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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

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

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers allsearchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search reportcovers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

[ ] The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

[ ] No protest accompanied the payment of additional search fees.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6567

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2008/000053

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006103116 A	05-10-2006	EP 1868643 A1	26-12-2007
EP 1172378 A	16-01-2002	NONE	
US 2006235207 A1	19-10-2006	AU 2003284427 A1	18-06-2004
		EP 1571211 A1	07-09-2005
		WO 2004048571 A1	10-06-2004
WO 2005018424 A	03-03-2005	NONE	

Form PCT/ISA/210 (patent family annex) (April 2005)

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
11 December 2008 (11.12.2008)

PCT

(10) International Publication Number
WO 2008/150946 A1

(51) International Patent Classification:
C07K 16/18 (2006.01)

(21) International Application Number:
PCT/US2008/065199

(22) International Filing Date: 30 May 2008 (30.05.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/940,931 30 May 2007 (30.05.2007) US

(71) Applicants *(for all designated States except US)*: **ABBOTT LABORATORIES** [US/US]; 100 Abbott Park Road, Abbott Park, Illinois 60064 (US). **ABBOTT GMBH & CO. KG** [DE/DE]; Knollstrabe, 67061 Ludwigshafen (DE).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **HILLEN, Heinz** [DE/DE]; Max-Planck-Str 17, 67454 Hassloch (DE). **BARGHORN, Stefan** [DE/DE]; Berliner Strasse 30, 68161 Mannheim (DE). **LABKOVSKY, Boris** [US/US]; P.O. Box 7, 115 Union Road, Wales, Massachusetts 01081 (US). **EBERT, Ulrich** [DE/DE]; Koenigstuhlstr 6, 68163 Mannheim (DE). **STRIEBINGER, Andreas R.** [DE/DE]; Ziegelofenweg 46A, 67346 Speyer (DE). **KELLER, Patrick** [DE/DE]; BernhardstraBe 30, 64291 Darmstadt (DE). **HINTON, Paul R.** [US/US]; 321 Dunsmuir Terrace, #4, Sunnyvale, California 94085 (US). **JUAN, Veronica M.** [US/US]; 1039 Laurel Street, Apt. #1, Menlo Park, California 94025 (US).

(74) Agent: **BECKER, Cheryl L.**; Abbott Laboratories, Dept. 0377, Bldg. AP6A-1A, 100 Abbott Park Road, Abbott Park, Illinois 60064-6008 (US).

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

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

Published:
— *with international search report*

(54) Title: HUMANIZED ANTIBODIES WHICH BIND TO Aβ (1-42) GLOBULOMER AND USES THEREOF

(57) Abstract: The present invention relates to binding proteins and, in particular, humanized antibodies that may be used, for example, in the diagnosis, treatment and prevention of Alzheimer's Disease and related conditions.

WO 2008/150946 A1

HUMANIZED ANTIBODIES WHICH BIND TO Aß(1-42) GLOBULOMER AND USES THEREOF

REFERENCE TO JOINT RESEARCH AGREEMENT

Contents of this application are under a joint research agreement entered into by and between Protein Design Labs, Inc. and Abbott Laboratories on August 31, 2006, and directed to humanized amyloid beta antibodies.

CROSS-REFERENCE TO RELATED APPLICATIONS

The subject application relates to International Appln. No. PCT/EP2006/011530 filed on November 30, 2006.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention relates to antibodies that may be used, for example, in the diagnosis, treatment and prevention of conditions such as amyloidoses (e.g., Alzheimer's Disease) and related conditions.

Background Information

Alzheimer's Disease (AD) is a neurodegenerative disorder characterized by a progressive loss of cognitive abilities and by characteristic neuropathological features comprising amyloid deposits, neurofibrillary tangles and neuronal loss in several regions of the brain (*see* Hardy and Selkoe (*Science* 297, 353 (2002); Mattson *Nature* 431, 7004 (2004). The principal constituents of amyloid deposits are amyloid beta-peptides (Aβ), with the 42 amino acid-long type (Aβ1-42) being the most prominent.

In particular, amyloid β(1-42) protein is a polypeptide having 42 amino acids which is derived from the amyloid precursor protein (APP) by proteolytic processing. This also

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6570

includes, in addition to human variants, isoforms of the amyloid β(1-42) protein present in organisms other than humans, in particular, other mammals, especially rats. This protein, which tends to polymerize in an aqueous environment, may be present in very different molecular forms.

A simple correlation of the deposition of insoluble protein with the occurrence or progression of dementia disorders such as, for example, Alzheimer's disease, has proved to be unconvincing (Terry et al., *Ann. Neurol.* 30. 572-580 (1991); Dickson et al., *Neurobiol. Aging* 16, 285-298 (1995)). In contrast, the loss of synapses and cognitive perception seems to correlate better with soluble forms of Aβ(1-42)(Lue et al., *Am. J. Pathol.* 155, 853-862 (1999); McLean et al., *Ann. Neurol.* 46, 860-866 (1999)).

Although polyclonal and monoclonal antibodies have been raised in the past against Aβ(1-42), none have proven to produce the desired therapeutic effect without also causing serious side effects in animals and/or humans. For example, passive immunization results from preclinical studies in very old APP23 mice which received a N-terminal directed anti-Aβ(1-42) antibody once weekly for 5 months indicate therapeutically relevant side effects. In particular, these mice showed an increase in number and severity of microhemorrhages compared to saline-treated mice (Pfeifer et al., *Science* 2002 298:1379). A similar increase in hemorrhages was also described for very old (>24 months) Tg2576 and PDAPP mice (Wilcock et al., *J Neuroscience* 2003, 23: 3745-51; Racke et al., *J Neuroscience* 2005, 25:629-636). In both strains, injection of anti-Aβ(1-42) resulted in a significant increase of microhemorrhages. Thus, a tremendous, unmet therapeutic need exists for the development of biologics that prevent or slow down the progression of the disease without inducing negative and potentially lethal effects on the human body. Such a need is particularly evident in view of the increasing longevity of the general population and, with this increase, an associated rise in the number of patents annually diagnosed

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6571

with Alzheimer's Disease or related disorders. Further, such antibodies will allow for proper diagnosis of Alzheimer's Disease in a patient experiencing symptoms thereof, a diagnosis which can only be confirmed upon autopsy at the present time. Additionally, the antibodies will allow for the elucidation of the biological properties of the proteins and other biological factors responsible for this debilitating disease.

All patents and publications referred to herein are hereby incorporated in their entirety by reference.

SUMMARY OF THE INVENTION

The present invention pertains to binding proteins, particularly antibodies (such as the one referred to herein by the terms "8F5" for the mouse/murine monoclonal antibody and "8F5hum8" for the humanized 8F5) capable of binding to soluble oligomers and, in particular, Aß(1-42) globulomer present in the brain of a patient having Alzheimer's Disease. For purposes herein, these binding proteins and, in particular, antibodies will be referred to as "globulomer-epitope specific antibodies". This means that the antibodies bind to one or more epitopes (e.g., the (1-42) amino acid region of the Aß(1-42) peptide) of the globulomer or antigen thought to be the cause of Alzeimer's disease. Further, the present invention also provides methods of producing and using these binding proteins or portions thereof.

One aspect of this invention pertains to a binding protein (e.g., antibody) comprising an antigen binding domain capable of binding to an Aß(1-42) globulomer. In one embodiment, the antigen-binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of:

CDR-VH1. X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:5), wherein:
X_1 is S;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6572

X2 is Y;
X3 is G;
X4 is M; and
X5 is S.

CDR-VH2. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17} (SEQ ID NO:6), wherein:
X_1 is S;
X_2 is I;
X_3 is N;
X_4 is S;
X_5 is N;
X_6 is G;
X_7 is G;
X_8 is S;
X_9 is T;
X_{10} is Y;
X_{11} is Y;
X_{12} is P;
X_{13} is D;
X_{14} is S;
X_{15} is V;
X_{16} is K; and
X_{17} is G.

CDR-VH3. X_1-X_2-X_3-X_4 (SEQ ID NO:7), wherein:
X_1 is S;
X_2 is G;
X_3 is D; and
X_4 is Y.

CDR-VL1. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16} (SEQ ID NO:8), wherein:
X_1 is R;
X_2 is S;
X_3 is S;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6573

X_4 is Q;
X_5 is S;
X_6 is L;
X_7 is V;
X_8 is Y;
X_9 is S;
X_{10} is N;
X_{11} is G;
X_{12} is D;
X_{13} is T;
X_{14} is Y;
X_{15} is L; and
X_{16} is H.

CDR-VL2. X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:9), wherein;
X_1 is K;
X_2 is V;
X_3 is S;
X_4 is N;
X_5 is R;
X_6 is F; and
X_7 is S.

and

CDR-VL3. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:10), wherein:
X_1 is S;
X_2 is Q;
X_3 is S;
X_4 is T;
X_5 is H;
X_6 is V;
X_7 is P;
X_8 is W; and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6574

X_9 is T.

Preferably, the antigen binding domain comprises at least one CDR comprising an amino acid sequence selected from the group consisting of residues 31-35 (i.e., SYGMS (SEQ ID NO:11); 8F5 VH CDR1) of SEQ ID NO.:1; residues 50-66 (i.e., SINSNGGSTYYPDSVKG (SEQ ID NO:12); 8F5 VH CDR2) of SEQ ID NO.:1; residues 98-108 (i.e., SGDY (SEQ ID NO:13); 8F5 VH CDR3) of SEQ ID NO.:1; residues 24-39 (i.e., RSSQSLVYSNGDTYLH (SEQ ID NO:14); 8F5 VL CDR1) of SEQ ID NO.:2; residues 55-61 (i.e., KVSNRFS (SEQ ID NO:15); 8F5 VL CDR2) of SEQ ID NO.:2; and residues 94-102 (i.e., SQSTHVPWT (SEQ ID NO:16); 8F5 VL CDR3) of SEQ ID NO.:2. In a preferred embodiment, the binding protein comprises at least 3 CDRs selected from the group consisting of the sequences disclosed above. More preferably, the 3 CDRs selected are from sets of variable domain CDRs selected from the group consisting of:

VH 8F5 CDR Set	
VH 8F5 CDR-H1	Residues 31-35 of SEQ ID NO.:1
VH 8F5 CDR-H2	Residues 50-66 of SEQ ID NO.:1
VH 8F5 CDR-H3	Residues 98-101 of SEQ ID NO.:1
VL 8F5 CDR Set	
VL 8F5 CDR-L1	Residues 24-39 of SEQ ID NO.:2
VL 8F5 CDR-L2	Residues 55-61 of SEQ ID NO.:2
VL 8F5 CDR-L3	Residues 94-102 of SEQ ID NO.:2

In one embodiment, the binding protein of the invention comprises at least two variable domain CDR sets. More preferably, the two variable domain CDR sets are: VH 8F5 CDR Set & VL 8F5 CDR Set. In another embodiment the binding protein disclosed above further comprises a human acceptor framework. Preferably the human acceptor framework comprises an amino acid sequence selected from the group consisting of: EVQLLESGGGLVQPGGSLRLSCAASGFTFS (SEQ ID NO:17); WVRQAPGKGLEWVS (SEQ ID NO:18); RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA (SEQ ID NO:19); WGQGTLVTVSS (SEQ ID NO:20); DIVMTQSPLSLPVTPGEPASISC (SEQ ID NO:21); WYLQKPGQSPQLLIY (SEQ ID NO:22);

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6575

GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC (SEQ ID NO:23); and FGGGTKVEIKR (SEQ ID NO:24).

In a preferred embodiment, the binding protein is a CDR grafted antibody or antigen binding portion thereof capable of binding to an Aß(1-42) globulomer. Preferably, the CDR grafted antibody or antigen binding portion thereof comprises one or more CDRs disclosed above. More preferably, the CDR grafted antibody or antigen binding portion thereof comprises at least one variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO:25 and SEQ ID NO:26. Most preferably, the CDR grafted antibody or antigen binding portion thereof comprises two variable domains selected from the group disclosed above. Preferably, the CDR grafted antibody or antigen binding portion thereof comprises a human acceptor framework. More preferably the human acceptor framework is any one of the human acceptor frameworks disclosed above.

In a preferred embodiment, the binding protein is a humanized antibody or antigen binding portion thereof capable of binding an Aß(1-42) globulomer. Preferably, the humanized antibody or antigen binding portion thereof comprises one or more CDRs disclosed above incorporated into a human antibody variable domain of a human acceptor framework. Preferably, the human antibody variable domain is a consensus human variable domain. More preferably, the human acceptor framework comprises at least one Framework Region amino acid substitution at a key residue, wherein the key residue is selected from the group consisting of a residue adjacent to a CDR; a glycosylation site residue; a rare residue; a residue capable of interacting with an Aß (1-42) globulomer; a residue capable of interacting with a CDR; a canonical residue; a contact residue between heavy chain variable region and light chain variable region; a residue within a Vernier zone; and a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework. Preferably, the human acceptor framework

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6576

human acceptor framework comprises at least one Framework Region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprises at least 52 amino acid residues identical to said human acceptor framework.

In a preferred embodiment, the binding protein is a humanized antibody or antigen binding portion thereof capable of binding to an Aß(1-42) globulomer. Preferably the humanized antibody, or antigen binding portion, thereof comprises one or more CDRs disclosed above. More preferably the humanized antibody, or antigen binding portion thereof, comprises three or more CDRs disclosed above. Most preferably the humanized antibody, or antigen-binding portion thereof, comprises six CDRs disclosed above.

In another embodiment of the claimed invention, the humanized antibody or antigen binding portion thereof comprises at least one variable domain having an amino acid sequence selected from the group consisting of SEQ ID NO:1 and SEQ ID NO.:2. With respect to SEQ ID NO:1 (8F5hum8 VL), based upon Kabat numbering, amino acid position 19 may be K; position 40 may be T; position 42 may be D; position 44 may be R; position 82A may be S; position 83 may be K; position 84 may be S; position 89 may be M, and J segment 107-109 "TLV" may be "STL". In connection with SEQ ID NO:2 (8F5hum7 VH), based upon Kabat numbering, amino acid position 7 may be T; position 14 may be S; position 15 may be L; position 17 may be D; position 18 may be Q; position 83 may be L; and position 87 may be F. More preferably, the humanized antibody or antigen-binding portion thereof comprises two variable domains selected from the group disclosed above. Most preferably, humanized antibody, or an antigen-binding portion thereof, comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of SEQ ID NOS.:11, 12 and 13 & SEQ ID NOS.:14, 15 and 16.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6577

In a preferred embodiment, the binding protein disclosed above comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG1 constant domain, a human IgG2 constant domain, a human IgG3 constant domain, a human IgG4 constant domain, a human IgE constant domain, and a human IgA constant domain. More preferably, the binding protein comprises SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28.

The binding protein of the invention is capable of binding Aß(1-42) globulomer. Preferably, the binding protein is a humanized antibody (e.g., 8F5) capable of modulating a biological function of an Aß(1-42) globulomer. More preferably the binding protein is capable of neutralizing an Aß(1-42) globulomer. Further, the humanized antibody binds with greater specificity to an amyloid beta (Aβ) protein globulomer than to an amyloid beta protein monomer. Thus, preferential binding is observed. The ratio of binding specificity to the globulomer versus the monomer is at least 1.4. In particular, the ratio is preferably at least about 1.4 to at least about 16.9. (A ratio of 1.0-17.5 including the endpoints) is also considered to fall within the scope of the present invention as well as decimal percentages thereof. For example, 1.1, 1.2, 1.3, …, 2.0, 2.1, 2.2…,17.1, 17.2, 17.3, 17.4, 17.5 as well as all full integers in between and percentages thereof are considered to fall within the scope of the present invention.) The amyloid beta protein monomer may be, for example, Aβ(1-42) monomer or Aβ(1-40) monomer.

In another embodiment, the binding protein of the invention has a dissociation constant (K_D) to an Aß(1-42) globulomer of $1x10^{-6}$ M to $1x10^{-12}$. Preferably, the antibody binds to an Aß(1-42) globulomer with high affinity, for instance, with a K_D of $1x10^{-7}$ M or greater affinity (for example, with a K_D of $3x10^{-}8$ M or greater affinity), with a K_D of $1x10^{-9}$ M or greater affinity (for example, $3x10^{-10}$ M or greater affinity), with a K_D or $1x10^{-10}$ M or greater affinity

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6578

(for example, with a K_D of $3x10^{-11}$ M or greater affinity) or with a K_D of $1x10^{-11}$ M or greater affinity.

One embodiment of the invention provides an antibody construct comprising any one of the binding proteins disclosed above and a linker polypeptide or an immunoglobulin. In a preferred embodiment, the antibody construct is selected from the group consisting of an immunoglobulin molecule, a monoclonal antibody, a chimeric antibody, a CDR-grafted antibody, a humanized antibody, a Fab, a Fab', a F(ab')2, a Fv, a disulfide linked Fv, a scFv, a single domain antibody, a diabody, a multispecific antibody, a dual specific antibody, a dual variable domain antibody and a bispecific antibody. In a preferred embodiment, the antibody construct comprises a heavy chain immunoglobulin constant domain selected from the group consisting of a human IgM constant domain, a human IgG1 constant domain, a human IgG2 constant domain, a human IgG3 constant domain, a human IgG4 constant domain, a human IgE constant domain, and a human IgA constant domain. More preferably, the antibody construct comprises (SEQ ID NO:25 and SEQ ID NO:26) or (SEQ ID NO:27 and SEQ ID NO:28). In another embodiment the invention provides an antibody conjugate comprising an the antibody construct disclosed above and an agent an agent selected from the group consisting of; an immunoadhension molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent. In a preferred embodiment the imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin. More preferably the imaging agent is a radiolabel selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm. In a preferred embodiment the therapeutic or cytotoxic agent is selected from the group consisting of; an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6579

In another embodiment, the antibody construct is glycosylated. Preferably, the glycosylation is a human glycosylation pattern.

In a further embodiment, the binding protein, antibody construct or antibody conjugate disclosed above exists as a crystal. Preferably, the crystal is a carrier-free pharmaceutical controlled release crystal. In a preferred embodiment, the crystallized binding protein, crystallized antibody construct or crystallized antibody conjugate has a greater half life in vivo than its soluble counterpart. In another preferred embodiment, the crystallized binding protein, crystallized antibody construct or crystallized antibody conjugate retains biological activity after crystallization.

One aspect of the invention pertains to an isolated nucleic acid encoding the binding protein, antibody construct or antibody conjugate disclosed above. A further embodiment provides a vector comprising the isolated nucleic acid disclosed above wherein said vector is selected from the group consisting of pcDNA; pTT (Durocher et al., *Nucleic Acids Research* 2002, Vol 30, No. 2); pTT3 (pTT with additional multiple cloning site; pEFBOS (Mizushima, S. and Nagata, S., (1990) *Nucleic Acids Research*, Vol. 18, No. 17); pBV; pJV; and pBJ.

The present invention also encompasses a host cell which is transformed with the vector disclosed above. Preferably, the host cell is a prokaryotic cell. More preferably, the host cell is *E. coli*. In a related embodiment, the host cell is an eukaryotic cell. Preferably, the eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell. More preferably, the host cell is a mammalian cell including, but not limited to, CHO and COS; or a fungal cell such as Saccharomyces cerevisiae; or an insect cell such as Sf9.

Additionally, the present invention includes a method of producing a binding protein that binds Aß(1-42) globulomer,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6580

comprising culturing any one of the host cells disclosed above in a culture medium under conditions and for a time sufficient to produce a binding protein that binds Aß(1-42). Another embodiment provides a binding protein produced according to the method disclosed above.

One embodiment provides a composition for the release of a binding protein, as defined herein, wherein the composition comprises a formulation which in turn comprises a crystallized binding protein, crystallized antibody construct or crystallized antibody conjugate as disclosed above and an ingredient; and at least one polymeric carrier. Preferably the polymeric carrier is a polymer selected from one or more of the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo)phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride-alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid, oligosaccharides, glycaminoglycans, sulfated polyeaccharides, blends and copolymers thereof. Preferably the ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-cyclodextrin, methoxypolyethylene glycol and polyethylene glycol. Another embodiment provides a method for treating a mammal comprising the step of administering to the mammal an effective amount of the composition disclosed above.

The invention also encompasses a pharmaceutical composition comprising a binding protein, antibody construct or antibody conjugate as disclosed above and a pharmaceutically acceptable carrier. In a further embodiment, the pharmaceutical composition comprises at least one additional therapeutic agent for treating a disorder in which

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6581

activity is detrimental. Preferably the additional therapeutic agent is selected from the group consisting of a cholesterinase inhibitor, a partial NMDA receptor blocker, a glycosaminoglycan mimetic, an inhibitor or allosteric modulator of gamma secretase, a luteinizing hormone blockade gonadotropin releasing hormone agonist, a serotinin 5-HT1A receptor antagonist, a chelating agent, a neuronal selective L-type calcium channel blocker, an immunomodulator, an amyloid fibrillogenesis inhibitor or amyloid protein deposition inhibitor, a PDE4 inhibitor, a histamine agonist, a receptor protein for advanced glycation end products, a PARP stimulator, a serotonin 6-receptor antagonist, a 5-HT4 receptor agonist, a human steroid, a glucose uptake stimulant which enhances neuronal metabolism, a selective CB1 antagonist, a partial agonist at benzodiazepine receptors, an amyloid beta production antagonist or inhibitor, an amyloid beta deposition inhibitor, a NNR alpha-7 partial antagonist, a therapeutic targeting PDE4, a RNA translation inhibitor, a muscarinic agonist, a nerve growth factor receptor agonist, a NGF receptor agonist and a gene therapy modulator.

In another aspect, the invention provides a method for inhibiting activity of Aß(1-42) globulomer comprising contacting Aß(1-42) globulomer with a binding protein disclosed above such that Aß(1-42) globulomer activity is inhibited. In a related aspect, the invention provides a method for inhibiting human Aß(1-42) globulomer activity in a human subject suffering from a disorder in which Aß(1-42) globulomer activity is detrimental, comprising administering to the human subject a binding protein disclosed above such that Aß(1-42) globulomer activity in the human subject is inhibited and treatment is achieved. Examples of conditions or diseases which may be treated using this method include but are not limited to Alpha1-antitrypsin-deficiency, C1-inhibitor deficiency angioedema, Antithrombin deficiency thromboembolic disease, Kuru, Creutzfeld-Jacob disease/scrapie, Bovine spongifor encephalopathy, Gerstmann-Straussler-Scheinker

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6582

disease, Fatal familial insomnia, Huntington's disease, Spinocerebellar ataxia, Machado-Joseph atrophy, Dentato-rubro-pallidoluysian atrophy, Frontotemporal dementia, Sickle cell anemia, Unstable hemoglobin inclusion-body hemolysis, Drug-induced inclusion body hemolysis, Parkinson's disease, Systemic AL amyloidosis, Nodular AL amyloidosis, Systemic AA amyloidosis, Prostatic amyloid, Hemodialysis amyloidosis, Hereditary (Icelandic) cerebral angiopathy, Huntington's disease, Familial visceral amyloid, Familial visceral polyneuropathy, Familial visceral amyloidosis, Senile systemic amyloidosis, Familial amyloid neurophathy, Familial cardiac amyloid, Alzheimer's disease, Down's syndrome, Medullary carcinoma thyroid and Type 2 diabetes mellitus (T2DM). Preferably the disorder is selected from an amyloidosis such as, for example, Alzheimer's Disease or Down's Syndrome.

In another aspect the invention provides a method of treating a patient suffering from a disorder in which Aß(1-42) globulomer is detrimental comprising the step of administering any one of the binding proteins disclosed above before, concurrent, or after the administration of a second agent, as discussed above. In a preferred embodiment, the second agent is selected from the group consisting of a small molecule or a biologic (i.e., see list of additional therapeutic agents presented above (e.g., an additional antibody, a cholinesterase inhibitor, a partial NMDA receptor blocker, etc.)).

In a preferred embodiment, the pharmaceutical compositions disclosed above are administered to the subject by at least one mode selected from parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6583

intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus, vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

One aspect of the invention provides at least one Aß(1-42) globulomer anti-idiotype antibody to at least one Aß(1-42) globulomer binding protein of the present invention. The anti-idiotype antibody includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule such as, but not limited to, at least one complementarily determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework region, or; any portion thereof, that can be incorporated into a binding protein of the present invention.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1(A) illustrates the nucleotide sequence (SEQ ID NO:3) of the variable heavy chain of humanized antibody 8F5, and Figure 1(B) illustrates the amino acid sequence of the variable heavy chain (SEQ ID NO:1) encoded by this nucleotide sequence. Figure 1(C) illustrates the nucleotide sequence (SEQ ID NO:4) of the variable light chain of humanized antibody 8F5, and Figure 1(D) illustrates the amino acid sequence (SEQ ID NO:2) of the variable light chain encoded by this nucleotide sequence. (All CDR regions are underlined.)

Figure 2(A) shows an SDS PAGE of standard proteins (molecular marker proteins, lane 1); Aβ(1-42) fibril preparation; control (lane 2); Aβ(1-42) fibril preparation + mAb 8F5hum8, 20h, 37°C, supernatant (lane 3); Aβ(1-42) fibril preparation + mAb 8F5hum8, 20h, 37°C, pellet (lane 4); Aβ(1-42) fibril preparation + mAb 6E10, 20h, 37°C, supernatant (lane 5); Aβ(1-42) fibril preparation + mAb 6E10, 20h 37°C, pellet (lane 6);

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6584

Aß (1-42) fibril preparation + mAb IgG2a, 20h 37°C, supernatant (lane 7); Aß (1-42) fibril preparation + mAb IgG2a, 20h 37°C, pellet (lane 8), and Figure 2(B) shows the results of the quantitative analysis of mAbs bound to Aβ-fibrils in percent of total antibody.

Figure 3 illustrates the binding of the biotinylated mouse 8F5 antibody to the Aß(1-42) globulomer. In particular, the binding of the biotinylated mouse 8F5 antibody is inhibited by increasing amounts of unlabeled mouse antibody(HYB) or humanized antibody (HUM8).

Figure 4 illustrates the alignment of the 8F5VH region amino acid sequences. The amino acid sequences of 8F5VH (SEQ ID NO:100), Hu8F5VHv1 (SEQ ID NO:101), Hu8F5VHv2 (SEQ ID NO:102), and the human YSE'CL (SEQ ID NO:103) and JH4 segments are shown in single letter code. The CDR sequences based on the definition of Kabat, E.A., et al. (1991) are underlined in the mouse 8F5VH sequence. The CDR sequences in the acceptor human VH segment are omitted in the figure. The single underlined amino acids in the Hu8F5VHv1 and Hu8F5VHv2 sequences are predicted to contact the CDR sequences, and therefore have been substituted with the corresponding mouse residues. The double underlined amino acids in the Hu8F5VHv1 and Hu8F5VHv2 sequences have been changed to the consensus amino acids in the same human VH subgroup to eliminate potential immunogenicity.

Figure 5 illustrates the alignment of the 8F5VL region amino acid sequences. The amino acid sequences of 8F5VL (SEQ ID NO:104), Hu8F5VL (SEQ ID NO:105), and the human TR1.37'CL (SEQ ID NO:106) and JK4 segments are shown in single letter code. The CDR sequences based on the definition of Kabat, E.A., et al. (1991) are underlined in the mouse 8F5VL sequence. The CDR sequences in the acceptor human VL segment are omitted in the figure. The single underlined amino acid in the Hu8F5VL

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6585

sequence is predicted to contact the CDR sequences, and therefore has been substituted with the corresponding mouse residue. The double underlined amino acids in the Hu8F5VL sequence have been changed to the consensus amino acids in the same human VL subgroup to eliminate potential immunogenicity.

Figure 6 illustrates the nucleotide sequence (SEQ ID NO:107) and deduced amino acid sequence (SEQ ID NO:108) of the heavy chain variable region of Hu8F5VHv1 in the mini exon. The signal peptide sequence is in italics. The mature heavy chain begins with a glutamate residue (indicated in bold). The CDRs based on the definition of Kabat, E.A., et al. (1991) are underlined. The sequence is flanked by unique MluI (ACGCGT) and XbaI (TCTAGA) sites.

Figure 7 illustrates the nucleotide sequence (SEQ ID NO:109) and deduced amino acid sequence (SEQ ID NO:110) of the heavy chain variable region of Hu8F5VHv2 in the mini exon. The signal peptide sequence is in italics. The mature heavy chain begins with a glutamate residue (indicated in bold). The CDRs based on the definition of Kabat, E.A., et al. (1991) are underlined. The sequence is flanked by unique MluI (ACGCGT) and XbaI (TCTAGA) sites.

Figure 8 illustrates the nucleotide sequence (SEQ ID NO:111) and deduced amino acid sequence (SEQ ID NO:112) of the light chain variable region of Hu8F5VL in the mini exon. The signal peptide sequence is in italics. The mature light chain begins with an aspartate residue (indicated in bold). The CDRs based on the definition of Kabat, E.A., et al. (1991) are underlined. The sequence is flanked by unique MluI (ACGCGT) and XbaI (TCTAGA) sites.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6586

Figure 9 illustrates the restriction maps of expression plasmids pHu8F5VHv1-Cg1 and pVk-Hu8F5VL.

Figure 10 illustrates the competition ELISA to compare the relative binding affinities of various 8F5 antibodies to human A-beta oligomer antigen (1-42). The binding of biotinylated Mu8F5 to immobilized human A-beta oligomer antigen (1-42) was analyzed in the presence of different amounts of Mu8F5 and Hu8F5 competitor antibodies as described in Example I.

DETAILED DESCRIPTION OF THE INVENTION

Unless otherwise defined herein, scientific and technical terms used in connection with the present invention shall have the meanings that are commonly understood by those of ordinary skill in the art. The meaning and scope of the terms should be clear; however, in the event of any latent ambiguity, definitions provided herein take precedent over any dictionary or extrinsic definition. Further, unless otherwise required by context, singular terms shall include pluralities and plural terms shall include the singular. In this application, the use of "or" means "and/or" unless stated otherwise. Furthermore, the use of the term "including", as well as other forms, such as "includes" and "included", is not limiting. Also, terms such as "element" or "component" encompass both elements and components comprising one unit and elements and components that comprise more than one subunit unless specifically stated otherwise.

Generally, nomenclatures used in connection with, and techniques of, cell and tissue culture, molecular biology, immunology, microbiology, genetics and protein and nucleic acid chemistry and hybridization described herein are those well known and commonly used in the art. The methods and techniques of the present invention are generally performed according to conventional methods well known in the art and as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6587

described in various general and more specific references that are cited and discussed throughout the present specification unless otherwise indicated. Enzymatic reactions and purification techniques are performed according to manufacturer's specifications, as commonly accomplished in the art or as described herein. The nomenclatures used in connection with, and the laboratory procedures and techniques of, analytical chemistry, synthetic organic chemistry, and medicinal and pharmaceutical chemistry described herein are those well known and commonly used in the art. Standard techniques are used for chemical syntheses, chemical analyses, pharmaceutical preparation, formulation, and delivery, and treatment of patients.

In order that the present invention may be more readily understood, select terms are defined below.

The term "polypeptide" as used herein, refers to any polymeric chain of amino acids. The terms "peptide" and "protein" are used interchangeably with the term polypeptide and also refer to a polymeric chain of amino acids. The term "polypeptide" encompasses native or artificial proteins, protein fragments and polypeptide analogs of a protein sequence. A polypeptide may be monomeric or polymeric.

The term "isolated protein" or "isolated polypeptide" is a protein or polypeptide that by virtue of its origin or source of derivation is not associated with naturally associated components that accompany it in its native state; is substantially free of other proteins from the same species; is expressed by a cell from a different species; or does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6588

The term "recovering" as used herein, refers to the process of rendering a chemical species such as a polypeptide substantially free of naturally associated components by isolation, e.g., using protein purification techniques well known in the art.

The term "Aβ(X-Y)" herein refers to the amino acid sequence from amino acid position "X" to amino acid position "Y" of the human amyloid β protein including both X and Y and, in particular, refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA (SEQ ID NO:29) or any of its naturally occurring variants, in particular, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start position of the Aβ peptide, including both position X and position Y or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation. An "additional" amino acid substitution is defined herein as any deviation from the canonical sequence that is not found in nature.

More specifically, the term "Aβ(1-42)" herein refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the human amyloid β protein including both 1 and 42 and, in particular, refers to the amino acid sequence from amino acid position 1 to amino acid position 42 of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA (i.e., the full sequence corresponding to amino acid positions 1 to 42; SEQ ID NO:29) or any of its naturally occurring variants. Such variants may be, for example, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), D23N ("Iowa"), A42T and A42V wherein the numbers are relative to the start of the Aβ

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6589

peptide, including both 1 and 42 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation. Likewise, the term "Aβ(1-40)" herein refers to the amino acid sequence from amino acid position 1 to amino acid position 40 of the human amyloid β protein including both 1 and 40 and refers, in particular, to the amino acid sequence from amino acid position 1 to amino acid position 40 of the amino acid sequence DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV (SEQ ID NO:30) or any of its naturally occurring variants. Such variants include, for example, those with at least one mutation selected from the group consisting of A2T, H6R, D7N, A21G ("Flemish"), E22G ("Arctic"), E22Q ("Dutch"), E22K ("Italian"), and D23N ("Iowa") wherein the numbers are relative to the start position of the Aβ peptide, including both 1 and 40 or a sequence with up to three additional amino acid substitutions none of which may prevent globulomer formation.

The term "Aβ(X-Y) globulomer" (also known as "Aβ(X-Y) globular oligomer") herein refers to a soluble, globular, non-covalent association of Aβ(X-Y) peptides, as defined above, possessing homogeneity and distinct physical characteristics. The Aβ(X-Y) globulomers are stable, non-fibrillar, oligomeric assemblies of Aβ(X-Y) peptides which are obtainable by incubation with anionic detergents. In contrast to monomer and fibrils, these globulomers are characterized by defined assembly numbers of subunits (e.g., early assembly forms, n=3-6, oligomers A", and late assembly forms, n=12-14, " oligomers B", as described in International Application Publication No. WO 04/067561 herein incorporated by reference). The globulomers have a 3-dimensional globular type structure ("molten globule", *see* Barghorn et al., 2005, *J Neurochem*, 95, 834-847). They may be further characterized by one or more of the following features:

- cleavability of N-terminal amino acids X-23 with promiscuous proteases (such as thermolysin or endoproteinase GluC) yielding truncated forms Aß(X-Y) globulomers;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6590

- non-accessibility of C-terminal amino acids 24-Y with promiscuous proteases and antibodies; and
- truncated forms of these Aß(X-Y) globulomers maintain the 3-dimensional core structure of the globulomers with a better accessibility of the core epitope Aß(20-Y) in its globulomer conformation.

According to the invention and, in particular, for the purpose of assessing the binding affinities of the antibodies of the present invention, the term "Aβ(X-Y) globulomer" herein refers to a product which is obtainable by a process as described in International Application Publication No. WO 04/067561, which is incorporated herein in its entirety by reference. The process comprises unfolding a natural, recombinant or synthetic Aβ (X-Y) peptide or a derivative thereof; exposing the at least partially unfolded Aβ (X-Y) peptide or derivative thereof to a detergent, reducing the detergent action and continuing incubation.

For the purpose of unfolding the peptide, hydrogen bond-breaking agents such as, for example, hexafluoroisopropanol (HFIP) may be allowed to act on the protein. Times of action of a few minutes, for example about 10 to 60 minutes, are sufficient when the temperature of action is from about 20 to 50°C and, in particular, about 35 to 40°C. Subsequent dissolution of the residue evaporated to dryness, preferably in concentrated form, in suitable organic solvents miscible with aqueous buffers such as, for example, dimethyl sulfoxide (DMSO), results in a suspension of the at least partially unfolded peptide or derivative thereof which can be used subsequently. If required, the stock suspension may be stored at low temperature, for example, at about -20°C for an interim period.

Alternatively, the peptide or the derivative thereof may be taken up in slightly acidic, preferably aqueous, solution, for example, a solution of about 10 mM aqueous HCl. After an incubation time of approximately a few minutes, insoluble

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6591

components are removed by centrifugation. A few minutes at 10,000 g is expedient. These method steps are preferably carried out at room temperature, i.e., a temperature in the range of from 20 to 30°C. The supernatant obtained after centrifugation contains the Aβ (X-Y) peptide or a derivative thereof and may be stored at low temperature, for example at about -20°C, for an interim period.

The following exposure to a detergent relates to oligomerization of the peptide or the derivative thereof to give the intermediate type of oligomers (in International Application Publication No. WO 04/067561 referred to as oligomers A). For this purpose, a detergent is allowed to act on the, optionally, at least partially unfolded peptide or derivative thereof until sufficient intermediate oligomer has been produced. Preference is given to using ionic detergents, in particular, anionic detergents.

According to a particular embodiment, a detergent of the formula (I):

R-X,

is used, in which the radical "R" is unbranched or branched alkyl having from 6 to 20 and preferably 10 to 14 carbon atoms or unbranched or branched alkenyl having from 6 to 20 and preferably 10 to 14 carbon atoms, and the radical "X" is an acidic group or salt thereof with X being preferably selected from among $-COO^-M^+$, $-SO_3^-M^+$ and is, most preferably, $-OSO_3^-M^+$, and M^+ is a hydrogen cation or an inorganic or organic cation preferably selected from alkali metal cations, alkaline earth metal cations and ammonium cations. Most advantageous are detergents of the formula (I) in which R is an unbranched alkyl of which alk-1-yl radicals must be mentioned, in particular. Particular preference is given to sodium dodecyl sulfate (SDS). Lauric acid and oleic acid can also be used advantageously. The sodium salt of the detergent lauroylsarcosin (also known as sarkosyl NL-30 or Gardol®) is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6592

also particularly advantageous.

The time of detergent action, in particular, depends on whether, and if yes, to what extent the peptide or derivative thereof subjected to oligomerization has unfolded. If, according to the unfolding step, the peptide or derivative thereof has been treated beforehand with a hydrogen bond-breaking agent (i.e., in particular with hexafluoroisopropanol), times of action in the range of a few hours, advantageously from about 1 to 20 and, in particular, from about 2 to 10 hours, are sufficient when the temperature of action is about 20 to 50°C and, in particular, from about 35 to 40°C. If a less unfolded or an essentially not unfolded peptide or derivative thereof is the starting point, correspondingly longer times of action are expedient. If the peptide or derivative thereof has been pretreated, for example, according to the procedure indicated above as an alternative to the HFIP treatment or said peptide or derivative thereof is directly subjected to oligomerization, times of action in the range from about 5 to 30 hours and, in particular, from about 10 to 20 hours are sufficient when the temperature of action is from about 20 to 50°C and, in particular, from about 35 to 40°C. After incubation, insoluble components are advantageously removed by centrifugation. A few minutes at 10,000 g is expedient.

The detergent concentration to be chosen depends on the detergent used. If SDS is used, a concentration in the range from 0.01 to 1% by weight, preferably, from 0.05 to 0.5% by weight, for example, of about 0.2% by weight, proves expedient. If lauric acid or oleic acid is used, somewhat higher concentrations are expedient, for example, in a range from 0.05 to 2% by weight, preferably, from 0.1 to 0.5% by weight, for example, of about 0.5% by weight.

The detergent action should take place at a salt concentration approximately in the physiological range. Thus, in particular NaCl concentrations in the range from 50 to 500 mM,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6593

preferably, from 100 to 200 mM and, more particularly, at about 140 mM are expedient.

The subsequent reduction of the detergent action and continuation of incubation relates to further oligomerization to give the Aβ(X-Y) globulomer of the invention (in International Application Publication No. WO 04/067561 referred to as oligomer B). Since the composition obtained from the preceding step regularly contains detergent and a salt concentration in the physiological range, it is then expedient to reduce detergent action and, preferably, also salt concentration. This may be carried out by reducing the concentration of detergent and salt, for example, by diluting expediently with water or a buffer of lower salt concentration, for example, Tris-HCl, pH 7.3. Dilution factors in the range from about 2 to 10, advantageously, in the range from about 3 to 8 and, in particular, of about 4, have proved suitable. The reduction in detergent action may also be achieved by adding substances which can neutralize this detergent action. Examples of these include substances capable of complexing the detergents, like substances capable of stabilizing cells in the course of purification and extraction measures, for example, particular EO/PO block copolymers, in particular, the block copolymer under the trade name Pluronic® F 68. Alkoxylated and, in particular, ethoxylated alkyl phenols such as the ethoxylated t-octylphenols of the Triton® X series, in particular, Triton® X100, 3-(3-cholamidopropyldimethylammonio)-1-propanesulfonate (CHAPS®) or alkoxylated and, in particular, ethoxylated sorbitan fatty esters such as those of the Tween® series, in particular, Tween® 20, in concentration ranges around or above the particular critical micelle concentration, may be equally used.

Subsequently, the solution is incubated until sufficient Aβ(X-Y) globulomer has been produced. Times of action in the range of several hours, preferably, in the range from about 10 to 30 hours and, in particular, in the range from about 15 to

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6594

25 hours, are sufficient when the temperature of action is about 20 to 50°C and, in particular, about 35 to 40°C. The solution may then be concentrated and possible residues may be removed by centrifugation. Again, a few minutes at 10,000 g proves expedient. The supernatant obtained after centrifugation contains an Aβ(X-Y) globulomer as described herein.

An Aβ(X-Y) globulomer can be finally recovered, e.g., by ultrafiltration, dialysis, precipitation or centrifugation. It is further preferred if electrophoretic separation of the Aβ(X-Y) globulomers under denaturing conditions, e.g. by SDS-PAGE, produces a double band (e.g., with an apparent molecular weight of 38/48 kDa for Aβ(1-42)) and especially preferred if upon glutardialdehyde treatment of the oligomers, before separation, these two bands are merged into one. It is also preferred if size exclusion chromatography of the globulomers results in a single peak (e.g., corresponding to a molecular weight of approximately 60 kDa for Aβ(1-42)). Starting from Aβ(1-42) peptide, the process is, in particular, suitable for obtaining Aβ(1-42) globulomers. Preferably, the globulomer shows affinity to neuronal cells and also exhibits neuromodulating effects. A "neuromodulating effect" is defined as a long-lasting inhibitory effect of a neuron leading to a dysfunction of the neuron with respect to neuronal plasticity.

According to another aspect of the invention, the term "Aβ(X-Y) globulomer" herein refers to a globulomer consisting essentially of Aβ(X-Y) subunits, wherein it is preferred if, on average, at least 11 of 12 subunits are of the Aβ(X-Y) type, more preferred, if less than 10% of the globulomers comprise any non-Aβ(X-Y) peptides and, most preferred, if the content of non-Aβ(X-Y) peptides in the preparation is below the detection threshold. More specifically, the term "Aβ(1-42) globulomer" herein refers to a globulomer comprising Aβ(1-42) units as defined above; the term "Aβ(12-42) globulomer" herein refers to a globulomer comprising Aβ(12-42) units as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6595

defined above; and the term "Aβ(20-42) globulomer" herein refers to a globulomer comprising Aβ(20-42) units as defined above.

The term "cross-linked Aβ(X-Y) globulomer" herein refers to a molecule obtainable from an Aβ(X-Y) globulomer as described above by cross-linking, preferably, chemically cross-linking, more preferably, aldehyde cross-linking and, most preferably, glutardialdehyde cross-linking of the constituent units of the globulomer. In another aspect of the invention, a cross-linked globulomer is essentially a globulomer in which the units are at least partially joined by covalent bonds, rather than being held together by non-covalent interactions only.

The term "Aβ(X-Y) globulomer derivative" herein refers, in particular, to a globulomer that is labelled by being covalently linked to a group that facilitates detection, preferably, a fluorophore, e.g., fluorescein isothiocyanate, phycoerythrin, Aequorea victoria fluorescent protein, Dictyosoma fluorescent protein or any combination or fluorescence-active derivatives thereof; a chromophore; a chemoluminophore, e.g., luciferase, preferably Photinus pyralis luciferase, Vibrio fischeri luciferase, or any combination or chemoluminescence-active derivatives thereof; an enzymatically active group, e.g., peroxidase such as horseradish peroxidase, or an enzymatically active derivative thereof; an electron-dense group, e.g., a heavy metal containing group such as a gold containing group; a hapten, e.g., a phenol derived hapten; a strongly antigenic structure, e.g., peptide sequence predicted to be antigenic such as by the algorithm of Kolaskar and Tongaonkar; an aptamer for another molecule; a chelating group, e.g., hexahistidinyl; a natural or nature-derived protein structure mediating further specific protein-protein interactions, e.g., a member of the fos/jun pair; a magnetic group, e.g., a ferromagnetic group; or a radioactive group such as a group comprising ^{1}H, ^{14}C, ^{32}P, ^{35}S or ^{125}I or any combination thereof; or to a globulomer

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6596

flagged by being covalently or by non-covalently linked by high-affinity interaction, preferably, covalently linked to a group that facilitates inactivation, sequestration, degradation and/or precipitation, preferably, flagged with a group that promotes *in vivo* degradation, more preferably, with ubiquitin, where it is particularly preferred if this flagged oligomer is assembled *in vivo*; or to a globulomer modified by any combination of the above. Such labelling and flagging groups and methods for attaching them to proteins are known in the art. Labelling and/or flagging may be performed before, during or after globulomerization. In another aspect of the invention, a globulomer derivative is a molecule obtainable from a globulomer by a labelling and/or flagging reaction. Correspondingly, the term "Aβ(X-Y) monomer derivative" herein refers, in particular, to an Aβ monomer that is labelled or flagged as described for the globulomer.

The term "greater affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer, on the one hand, and antibody-globulomer complex, on the other, is further in favor of the antibody-globulomer complex. Likewise, the term "smaller affinity" herein refers to a degree of interaction where the equilibrium between unbound antibody and unbound globulomer, on the one hand, and antibody-globulomer complex, on the other, is further in favor of the unbound antibody and unbound globulomer.

The term "Aβ(X-Y) monomer" herein refers to the isolated form of the Aβ(X-Y) peptide, preferably, a form of the Aβ(X-Y) peptide which is not engaged in essentially non-covalent interactions with other Aβ peptides. Practically, the Aβ(X-Y) monomer is usually provided in the form of an aqueous solution. Preferably, the aqueous monomer solution contains 0.05% to 0.2%, more preferably, about 0.1% NaOH when used, for instance, for determining the binding affinity of the antibody of the present invention. In another preferable situation, the aqueous monomer solution contains 0.05% to 0.2%, more

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6597

preferably, about 0.1% NaOH. When used, it may be expedient to dilute the solution in an appropriate manner. Further, it is usually expedient to use the solution within 2 hours, in particular, within 1 hour, and, especially, within 30 minutes after its preparation.

The term "fibril" herein refers to a molecular structure that comprises assemblies of non-covalently associated, individual Aβ(X-Y) peptides which show fibrillary structure under the electron microscope, which bind Congo red, exhibit birefringence under polarized light and whose X-ray diffraction pattern is a cross-β structure. The fibril may also be defined as a molecular structure obtainable by a process that comprises the self-induced polymeric aggregation of a suitable Aβ peptide in the absence of detergents, e.g., in 0.1 M HCl, leading to the formation of aggregates of more than 24, preferably, more than 100 units. This process is well known in the art. Expediently, Aβ(X-Y) fibril is used in the form of an aqueous solution. In a particularly preferred embodiment of the invention, the aqueous fibril solution is made by dissolving the Aβ peptide in 0.1% NH_4OH, diluting it 1:4 with 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4, followed by readjusting the pH to 7.4, incubating the solution at 37 °C for 20 h, followed by centrifugation at 10000 g for 10 min and resuspension in 20 mM NaH_2PO_4, 140 mM NaCl, pH 7.4.

The term "Aβ(X-Y) fibril" herein refers to a fibril comprising Aβ(X-Y) subunits where it is preferred if, on average, at least 90% of the subunits are of the Aβ(X-Y) type, more preferred, if at least 98% of the subunits are of the Aβ(X-Y) type and, most preferred, if the content of non-Aβ(X-Y) peptides is below the detection threshold.

Turning back to antibody 8F5, this Aβ(1-42) globulomer-specific antibody recognizes predominantly Aβ(1-42) globulomer forms and not standard preparations of Aβ(1-40) monomers, Aβ(1-42) monomers, Aβ-fibrils or sAPP (i.e, Aβ precursor) in contrast to, for example, competitor antibodies such as m266 and 3D6. Such specificity for globulomers is important

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6598

because specifically targeting the globulomer form of Aβ with a globulomer preferential antibody such as, for example, 8F5, will: 1) avoid targeting insoluble amyloid deposits, binding to which may account for inflammatory side effects observed during immunizations with insoluble Aβ; 2) spare Aβ monomer and APP that are reported to have precognitive physiological functions (Plan et al., *J. of Neuroscience* 23:5531-5535 (2003); and 3) increase the bioavailability of the antibody, as it would not be shaded or inaccessible through extensive binding to insoluble deposits.

The subject invention also includes isolated nucleotide sequences (or fragments thereof) encoding the variable light and heavy chains of antibody 8F5 as well as those nucleotide sequences (or fragments thereof) having sequences comprising, corresponding to, identical to, hybridizable to, or complementary to, at least about 70% (e.g., 70% 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78% or 79%), preferably at least about 80% (e.g., 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%), and more preferably at least about 90% (e.g, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%) identity to these encoding nucleotide sequences. (All integers (and portions thereof) between and including 70% and 100% are considered to be within the scope of the present invention with respect to percent identity.) Such sequences may be derived from any source (e.g., either isolated from a natural source, produced via a semi-synthetic route, or synthesized *de novo*). In particular, such sequences may be isolated or derived from sources other than described in the examples (e.g., bacteria, fungus, algae, mouse or human).

In addition to the nucleotide sequences described above, the present invention also includes amino acid sequences of the variable light and heavy chains of antibody 8F5 (or fragments of these amino acid sequences). Further, the present invention also includes amino acid sequences (or fragments thereof) comprising, corresponding to, identical to, or complementary to at least about 70% (e.g., 70%, 71%, 72%,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6599

73%, 74%, 75%, 76%, 77%, 78% or 79%), preferably at least about 80% (e.g., 80% 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88% or 89%), and more preferably at least about 90% identity (e.g., 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100%), to the amino acid sequences of the proteins of the present invention. (Again, all integers (and portions thereof) between and including 70% and 100% (as recited in connection with the nucleotide sequence identities noted above) are also considered to be within the scope of the present invention with respect to percent identity.)

For purposes of the present invention, a "fragment" of a nucleotide sequence is defined as a contiguous sequence of approximately at least 6, preferably at least about 8, more preferably at least about 10 nucleotides, and even more preferably at least about 15 nucleotides corresponding to a region of the specified nucleotide sequence.

The term "identity" refers to the relatedness of two sequences on a nucleotide-by-nucleotide basis over a particular comparison window or segment. Thus, identity is defined as the degree of sameness, correspondence or equivalence between the same strands (either sense or antisense) of two DNA segments (or two amino acid sequences). "Percentage of sequence identity" is calculated by comparing two optimally aligned sequences over a particular region, determining the number of positions at which the identical base or amino acid occurs in both sequences in order to yield the number of matched positions, dividing the number of such positions by the total number of positions in the segment being compared and multiplying the result by 100. Optimal alignment of sequences may be conducted by the algorithm of Smith & Waterman, Appl. Math. 2:482 (1981), by the algorithm of Needleman & Wunsch, J. Mol. Biol. 48:443 (1970), by the method of Pearson & Lipman, Proc. Natl. Acad. Sci. (USA) 85:2444 (1988) and by computer programs which implement the relevant algorithms (e.g., Clustal Macaw Pileup (http://cmgm.stanford.edu/biochem218/11Multiple.pdf; Higgins

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6600

et al., CABIOS. 5L151-153 (1989)), FASTDB (Intelligenetics), BLAST (National Center for Biomedical Information; Altschul et al., Nucleic Acids Research 25:3389-3402 (1997)), PILEUP (Genetics Computer Group, Madison, WI) or GAP, BESTFIT, FASTA and TFASTA (Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, Madison, WI). (See U.S. Patent No. 5,912,120.)

For purposes of the present invention, "complementarity" is defined as the degree of relatedness between two DNA segments. It is determined by measuring the ability of the sense strand of one DNA segment to hybridize with the anti-sense strand of the other DNA segment, under appropriate conditions, to form a double helix. A "complement" is defined as a sequence which pairs to a given sequence based upon the canonic base-pairing rules. For example, a sequence A-G-T in one nucleotide strand is "complementary" to T-C-A in the other strand.

In the double helix, adenine appears in one strand, thymine appears in the other strand. Similarly, wherever guanine is found in one strand, cytosine is found in the other. The greater the relatedness between the nucleotide sequences of two DNA segments, the greater the ability to form hybrid duplexes between the strands of the two DNA segments.

"Similarity" between two amino acid sequences is defined as the presence of a series of identical as well as conserved amino acid residues in both sequences. The higher the degree of similarity between two amino acid sequences, the higher the correspondence, sameness or equivalence of the two sequences. ("Identity between two amino acid sequences is defined as the presence of a series of exactly alike or invariant amino acid residues in both sequences.) The definitions of "complementarity", "identity" and "similarity" are well known to those of ordinary skill in the art.

"Encoded by" refers to a nucleic acid sequence which codes for a polypeptide sequence, wherein the polypeptide sequence or a portion thereof contains an amino acid sequence

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6601

of at least 3 amino acids, more preferably at least 8 amino acids, and even more preferably at least 15 amino acids from a polypeptide encoded by the nucleic acid sequence.

"Biological activity" as used herein, refers to all inherent biological properties of the Aβ(1-42) region of the globulomer. Such properties include, for example, the ability to bind to the 8F5 and functionally-related antibodies described herein.

The terms "specific binding" or "specifically binding", as used herein, in reference to the interaction of an antibody, a protein, or a peptide with a second chemical species, mean that the interaction is dependent upon the presence of a particular structure (e.g., an antigenic determinant or epitope) on the chemical species; for example, an antibody recognizes and binds to a specific protein structure rather than to proteins generally. If an antibody is specific for epitope "A", the presence of a molecule containing epitope A (or free, unlabeled A), in a reaction containing labeled "A" and the antibody, will reduce the amount of labeled A bound to the antibody.

The term "antibody", as used herein, broadly refers to any immunoglobulin (Ig) molecule comprised of four polypeptide chains, two heavy (H) chains and two light (L) chains, or any functional fragment, mutant, variant, or derivation thereof, which retains the essential epitope binding features of an Ig molecule. Such mutant, variant, or derivative anitbody formats are known in the art. Nonlimiting embodiments of which are discussed below.

In a full-length antibody, each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or VL) and a light chain constant region. The light chain constant region is comprised

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6602

of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Immunoglobulin molecules can be of any type (e.g., IgG, IgE, IgM, IgD, IgA and IgY), class (e.g., IgG 1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass.

The term "antigen-binding portion" of an antibody (or simply "antibody portion"), as used herein, refers to one or more fragments of an antibody that retain the ability to specifically bind to an antigen (*e.g.*, Aβ(1-42) globulomer). It has been shown that the antigen-binding function of an antibody can be performed by one or more fragments of a full-length antibody. Such antibody embodiments may also be bispecific, dual specific, or multi-specific, specifically binding to two or more different antigens. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the VH and CH1 domains; (iv) a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, (1989) *Nature* 341:544-546, Winter et al., Intern. Appln. Public. No. WO 90/05144 A1 herein incorporated by reference), which comprises a single variable domain; and (vi) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6603

VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see *e.g.*, Bird *et al.* (1988) *Science* 242:423-426; and Huston *et al.* (1988) *Proc. Natl. Acad. Sci. USA* 85:5879-5883). Such single chain antibodies are also encompassed herein within the term "antigen-binding portion" of an antibody. Other forms of single chain antibodies, such as diabodies, are also encompassed. Diabodies are bivalent, bispecific antibodies in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating two antigen binding sites (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). Such antibody binding portions are known in the art (Kontermann and Dubel eds., *Antibody Engineering* (2001) Springer-Verlag. New York. 790 pp. (ISBN 3-540-41354-5).

The term "antibody construct" as used herein refers to a polypeptide comprising one or more the antigen binding portions of the invention linked to a linker polypeptide or an immunoglobulin constant domain. Linker polypeptides comprise two or more amino acid residues joined by peptide bonds and are used to link one or more antigen binding portions. Such linker polypeptides are well known in the art (see *e.g.*, Holliger, P., *et al.* (1993) *Proc. Natl. Acad. Sci. USA* 90:6444-6448; Poljak, R.J., *et al.* (1994) *Structure* 2:1121-1123). An immunoglobulin constant domain refers to a heavy or light chain constant domain. Human IgG heavy chain and light chain constant domain amino acid sequences are known in the art and represented in Table 1.

TABLE 1: SEQUENCE OF HUMAN IgG HEAVY CHAIN CONSTANT DOMAIN AND LIGHT CHAIN CONSTANT DOMAIN

Protein	Sequence Identifier	Sequence
		123456789**0**123456789**0**123456789**0**12

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6604

Protein	Sequence Identifier	Sequence
		123456789**0**123456789**0**12345678**9****0**12
Ig gamma-1 constant region	**SEQ ID NO.:25**	ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
Ig gamma-1 constant region mutant	**SEQ ID NO.:26**	ASTKGPSVFPLAPSSKSTSGGTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFP AVLQSSGLYSLSSVVTVPSSSLGTQTY ICNVNHKPSNTKVDKKVEPKSCDKTHT CPPCPAPEAAGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPREEQYNSTYRVVSVL TVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTK NQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDK SRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
Ig Kappa constant region	**SEQ ID NO.:27**	TVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPREAKVQWKVDNALQSGNSQES VTEQDSKDSTYSLSSTLTLSKADYEKH KVYACEVTHQGLSSPVTKSFNRGEC
Ig Lambda constant region	**SEQ ID NO.:28**	QPKAAPSVTLFPPSSEELQANKATLVC LISDFYPGAVTVAWKADSSPVKAGVET TTPSKQSNNKYAASSYLSLTPEQWKSH RSYSCQVTHEGSTVEKTVAPTECS

Still further, an antibody or antigen-binding portion thereof may be part of a larger immunoadhesion molecule, formed by covalent or noncovalent association of the antibody or antibody portion with one or more other proteins or peptides. Examples of such immunoadhesion molecules include use of the streptavidin core region to make a tetrameric scFv molecule (Kipriyanov, S.M., *et al.* (1995) *Human Antibodies and Hybridomas* 6:93-101) and use of a cysteine residue, a marker peptide and a C-terminal polyhistidine tag to make bivalent

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6605

and biotinylated scFv molecules (Kipriyanov, S.M., *et al.* (1994) *Mol. Immunol.* 31:1047-1058). Antibody portions, such as Fab and $F(ab')_2$ fragments, can be prepared from whole antibodies using conventional techniques, such as papain or pepsin digestion, respectively, of whole antibodies. Moreover, antibodies, antibody portions and immunoadhesion molecules can be obtained using standard recombinant DNA techniques, as described herein.

An "isolated antibody", as used herein, is intended to refer to an antibody that is substantially free of other antibodies having different antigenic specificities (*e.g.*, an isolated antibody that specifically binds Aβ(1-42) globulomer is substantially free of antibodies that specifically bind antigens other than Aβ(1-42) globulomer). An isolated antibody that specifically binds Aβ(1-42) globulomer may, however, have cross-reactivity to other antigens, such as Aβ(1-42) globulomer molecules from other species. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs and in particular CDR3. However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The term "recombinant human antibody", as used herein, is intended to include all human antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies expressed using a recombinant expression vector transfected into a host cell (described below), antibodies

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6606

isolated from a recombinant, combinatorial human antibody library (Hoogenboom H.R., (1997) *TIB Tech.* 15:62-70; Azzazy H., and Highsmith W.E., (2002) *Clin. Biochem.* 35:425-445; Gavilondo J.V., and Larrick J.W. (2002) *BioTechniques* 29:128-145; Hoogenboom H., and Chames P. (2000) *Immunology Today* 21:371-378), antibodies isolated from an animal (e.g., a mouse) that is transgenic for human immunoglobulin genes (see e.g., Taylor, L. D., et al. (1992) Nucl. Acids Res. 20:6287-6295; Kellermann S-A., and Green L.L. (2002) *Current Opinion in Biotechnology* 13:593-597; Little M. et al (2000) *Immunology Today* 21:364-370) or antibodies prepared, expressed, created or isolated by any other means that involves splicing of human immunoglobulin gene sequences to other DNA sequences. Such recombinant human antibodies have variable and constant regions derived from human germline immunoglobulin sequences. In certain embodiments, however, such recombinant human antibodies are subjected to *in vitro* mutagenesis (or, when an animal transgenic for human Ig sequences is used, *in vivo* somatic mutagenesis) and thus the amino acid sequences of the VH and VL regions of the recombinant antibodies are sequences that, while derived from and related to human germline VH and VL sequences, may not naturally exist within the human antibody germline repertoire in vivo.

The term "chimeric antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species and constant region sequences from another species, such as antibodies having murine heavy and light chain variable regions linked to human constant regions.

The term "CDR-grafted antibody" refers to antibodies which comprise heavy and light chain variable region sequences from one species but in which the sequences of one or more of the CDR regions of VH and/or VL are replaced with CDR sequences of another species, such as antibodies having murine heavy and light chain variable regions in which one or more of the murine CDRs (*e.g.*, CDR3) has been replaced with human CDR sequences.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6607

The term "humanized antibody" refers to antibodies which comprise heavy and light chain variable region sequences from a non-human species (*e.g.*, a mouse) but in which at least a portion of the VH and/or VL sequence has been altered to be more "human-like", *i.e.*, more similar to human germline variable sequences. One type of humanized antibody is a CDR-grafted antibody, in which human CDR sequences are introduced into non-human VH and VL sequences to replace the corresponding nonhuman CDR sequences.

The terms "Kabat numbering", "Kabat definitions and "Kabat labeling" are used interchangeably herein. These terms, 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 an antigen binding portion thereof (Kabat *et al.* (1971) *Ann. NY Acad, Sci.* 190:382-391 and Kabat, E.A., *et al.* (1991) *Sequences of Proteins of Immunological Interest, Fifth Edition*, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For the heavy chain variable region, the hypervariable region ranges from amino acid positions 31 to 35 for CDR1, amino acid positions 50 to 65 for CDR2, and amino acid positions 95 to 102 for CDR3. For the light chain variable region, the hypervariable region ranges from amino acid positions 24 to 34 for CDR1, amino acid positions 50 to 56 for CDR2, and amino acid positions 89 to 97 for CDR3.

As used herein, the terms "acceptor" and "acceptor antibody" refer to the antibody or nucleic acid sequence providing or encoding at least 80%, at least 85%, at least 90%, at least 95%, at least 98% or 100% of the amino acid sequences of one or more of the framework regions. In some embodiments, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding the constant region(s). In yet another embodiment, the term "acceptor" refers to the antibody amino acid or nucleic acid sequence providing or encoding one or more of the framework

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6608

regions and the constant region(s). In a specific embodiment, the term "acceptor" refers to a human antibody amino acid or nucleic acid sequence that provides or encodes at least 80%, preferably, at least 85%, at least 90%, at least 95%, at least 98%, or 100% of the amino acid sequences of one or more of the framework regions. In accordance with this embodiment, an acceptor may contain at least 1, at least 2, at least 3, least 4, at least 5, or at least 10 amino acid residues that does (do) not occur at one or more specific positions of a human antibody. An acceptor framework region and/or acceptor constant region(s) may be, e.g., derived or obtained from a germline antibody gene, a mature antibody gene, a functional antibody (e.g., antibodies well-known in the art, antibodies in development, or antibodies commercially available).

As used herein, the term "CDR" refers to the complementarity determining region within antibody variable sequences. There are three CDRs in each of the variable regions of the heavy chain and the light chain, which are designated CDR1, CDR2 and CDR3, for each of the variable regions. The term “CDR set” as used herein refers to a group of three CDRs that occur in a single variable region capable of binding the antigen. The exact boundaries of these CDRs have been defined differently according to different systems. The system described by Kabat (Kabat et al., Sequences of Proteins of Immunological Interest (National Institutes of Health, Bethesda, MD (1987) and (1991)) not only provides an unambiguous residue numbering system applicable to any variable region of an antibody, but also provides precise residue boundaries defining the three CDRs. These CDRs may be referred to as Kabat CDRs. Chothia and coworkers (Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987) and Chothia et al., *Nature* 342:877-883 (1989)) found that certain sub- portions within Kabat CDRs adopt nearly identical peptide backbone conformations, despite having great diversity at the level of amino acid sequence. These sub-portions were designated as L1, L2 and L3 or H1, H2 and H3 where the "L" and the "H"

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6609

designates the light chain and the heavy chains regions, respectively. These regions may be referred to as Chothia CDRs, which have boundaries that overlap with Kabat CDRs. Other boundaries defining CDRs overlapping with the Kabat CDRs have been described by Padlan (*FASEB J.* 9:133-139 (1995)) and MacCallum (*J Mol Biol* 262(5):732-45 (1996)). Still other CDR boundary definitions may not strictly follow one of the above systems, but will nonetheless overlap with the Kabat CDRs, although they may be shortened or lengthened in light of prediction or experimental findings that particular residues or groups of residues or even entire CDRs do not significantly impact antigen binding. The methods used herein may utilize CDRs defined according to any of these systems, although preferred embodiments use Kabat or Chothia defined CDRs.

As used herein, the term "canonical" residue refers to a residue in a CDR or framework that defines a particular canonical CDR structure as defined by Chothia et al. (*J. Mol. Biol.* 196:901-907 (1987); Chothia et al., *J. Mol. Biol.* 227:799 (1992), both are incorporated herein by reference). According to Chothia et al., critical portions of the CDRs of many antibodies have nearly identical peptide backbone confirmations despite great diversity at the level of amino acid sequence. Each canonical structure specifies primarily a set of peptide backbone torsion angles for a contiguous segment of amino acid residues forming a loop.

As used herein, the terms "donor" and "donor antibody" refer to an antibody providing one or more CDRs. In a preferred embodiment, the donor antibody is an antibody from a species different from the antibody from which the framework regions are obtained or derived. In the context of a humanized antibody, the term "donor antibody" refers to a non-human antibody providing one or more CDRs.

As used herein, the term "framework" or "framework sequence" refers to the remaining sequences of a variable region minus the CDRs. Because the exact definition of a CDR sequence can be determined by different systems, the meaning

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6610

of a framework sequence is subject to correspondingly different interpretations. The six CDRs (CDR-L1, -L2, and -L3 of light chain and CDR-H1, -H2, and -H3 of heavy chain) also divide the framework regions on the light chain and the heavy chain into four sub-regions (FR1, FR2, FR3 and FR4) on each chain, in which CDR1 is positioned between FR1 and FR2, CDR2 between FR2 and FR3, and CDR3 between FR3 and FR4. Without specifying the particular sub-regions as FR1, FR2, FR3 or FR4, a framework region, as referred by others, represents the combined FR's within the variable region of a single, naturally occurring immunoglobulin chain. As used herein, a FR represents one of the four sub- regions, and FRs represents two or more of the four sub- regions constituting a framework region.

Human heavy chain and light chain acceptor sequences are known in the art. In one embodiment of the invention the human heavy chain and light chain acceptor sequences are selected from the sequences described below:

TABLE 2: HEAVY CHAIN ACCEPTOR SEQUENCES

SEQ ID No.	Protein region	Sequence
17	VH3-23/JH4 Fr1	EVQLLESGGGLVQPGGSLRLSCAASGFTFS
18	VH3-23/JH4 Fr2	WVRQAPGKGLEWVS
19	VH3-23/JH4 Fr3	RFTISRDNSKNTLYLQMNSLRAEDTAVYYCA
20	VH3-23/JH4 Fr4	WGQGTLVTVSS

TABLE 3: LIGHT CHAIN ACCEPTOR SEQUENCES

SEQ ID No.	Protein region	Sequence
21	A19/JK1 Fr1	DIVMTQSPLSLPVTPGEPASISC
22	A19/JK1 Fr2	WYLQKPGQSPQLLIY
23	A19/JK1 Fr3	GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYC
24	A19/JK1 Fr4	FGGGTKVEIKR

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6611

As used herein, the term "germline antibody gene" or "gene fragment" refers to an immunoglobulin sequence encoded by non-lymphoid cells that have not undergone the maturation process that leads to genetic rearrangement and mutation for expression of a particular immunoglobulin. (See, e.g., Shapiro et al., *Crit. Rev. Immunol.* 22(3): 183-200 (2002); Marchalonis et al., *Adv Exp Med Biol.* 484:13-30 (2001)). One of the advantages provided by various embodiments of the present invention stems from the recognition that germline antibody genes are more likely than mature antibody genes to conserve essential amino acid sequence structures characteristic of individuals in the species, hence less likely to be recognized as from a foreign source when used therapeutically in that species.

As used herein, the term "key" residues refer to certain residues within the variable region that have more impact on the binding specificity and/or affinity of an antibody, in particular a humanized antibody. A key residue includes, but is not limited to, one or more of the following: a residue that is adjacent to a CDR, a potential glycosylation site (can be either N- or O-glycosylation site), a rare residue, a residue capable of interacting with the antigen, a residue capable of interacting with a CDR, a canonical residue, a contact residue between heavy chain variable region and light chain variable region, a residue within the Vernier zone, and a residue in the region that overlaps between the Chothia definition of a variable heavy chain CDR1 and the Kabat definition of the first heavy chain framework.

As used herein, the term "humanized antibody" is an antibody or a variant, derivative, analog or fragment thereof which immunospecifically binds to an antigen of interest and which comprises a framework (FR) region having substantially the amino acid sequence of a human antibody and a complementary determining region (CDR) having substantially the amino acid sequence of a non-human antibody. As used herein, the term "substantially" in the context of a CDR

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6612

refers to a CDR having an amino acid sequence at least 80%, preferably at least 85%, more preferably at least 90%, more preferably at least 95%, more preferably at least 98% and most preferably at least 99% identical to the amino acid sequence of a non-human antibody CDR. A humanized antibody comprises substantially all of at least one, and typically two, variable domains (Fab, Fab', F(ab') 2, FabC, Fv) in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin (i.e., donor antibody) and all or substantially all of the framework regions are those of a human immunoglobulin consensus sequence. Preferably, a humanized antibody also comprises at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. In some embodiments, a humanized antibody contains both the light chain as well as at least the variable domain of a heavy chain. The antibody also may include the CH1, hinge, CH2, CH3, and CH4 regions of the heavy chain. In other embodiments, a humanized antibody only contains a humanized light chain. In some embodiments, a humanized antibody only contains a humanized heavy chain. In specific embodiments, a humanized antibody only contains a humanized variable domain of a light chain and/or humanized heavy chain.

The humanized antibody can be selected from any class of immunoglobulins, including IgM, IgG, IgD, IgA and IgE, and any isotype, including without limitation IgG 1, IgG2, IgG3 and IgG4. The humanized antibody may comprise sequences from more than one class or isotype, and particular constant domains may be selected to optimize desired effector functions using techniques well-known in the art.

The framework and CDR regions of a humanized antibody need not correspond precisely to the parental sequences, e.g., the donor antibody CDR or the consensus framework may be mutagenized by substitution, insertion and/or deletion of at least one amino acid residue so that the CDR or framework residue at that site does not correspond to either the donor antibody or the consensus framework. In a preferred

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6613

embodiment, such mutations, however, will not be extensive. Usually, at least 80%, preferably at least 85%, more preferably at least 90%, and most preferably at least 95% of the humanized antibody residues will correspond to those of the parental FR and CDR sequences. As used herein, the term "consensus framework" refers to the framework region in the consensus immunoglobulin sequence. As used herein, the term "consensus immunoglobulin sequence" refers to the sequence formed from the most frequently occurring amino acids (or nucleotides) in a family of related immunoglobulin sequences (See e.g., Winnaker, From Genes to Clones (Verlagsgesellschaft, Weinheim, Germany 1987). In a family of immunoglobulins, each position in the consensus sequence is occupied by the amino acid occurring most frequently at that position in the family. If two amino acids occur equally frequently, either can be included in the consensus sequence.

As used herein, "Vernier" zone refers to a subset of framework residues that may adjust CDR structure and fine-tune the fit to antigen as described by Foote and Winter (1992, *J. Mol. Biol.* 224:487-499, which is incorporated herein by reference). Vernier zone residues form a layer underlying the CDRs and may impact on the structure of CDRs and the affinity of the antibody.

As used herein, the term “neutralizing” refers to neutralization of biological activity of a globulomer when a binding protein specifically binds the globulomer. Preferably, a neutralizing binding protein is a neutralizing antibody whose binding to the Aβ(1-42) amino acid region of the globulomer results in inhibition of a biological activity of the globulomer. Preferably the neutralizing binding protein binds to the Aβ(1-42) region of the globulomer and reduces a biologically activity of the globulomer by at least about 20%, 40%, 60%, 80%, 85% or more. Inhibition of a biological activity of the globulomer by a neutralizing binding protein can be assessed by measuring one or more

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6614

indicators of globulomer biological activity well known in the art.

The term "activity" includes activities such as the binding specificity/affinity of an antibody for an antigen, for example, an anti-Aβ(1-42) antibody that binds to an Aβ(1-42) globulomer and/or the neutralizing potency of an antibody, for example, an anti-Aβ(1-42) antibody whose binding to Aβ(1-42) inhibits the biological activity of the globulomer.

The term "epitope" includes any polypeptide determinant capable of specific binding to an immunoglobulin or T-cell receptor. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl and, in certain embodiments, may have specific three-dimensional structural characteristics, and/or specific charge characteristics. An epitope is a region of an antigen that is bound by an antibody. In certain embodiments, an antibody is said to specifically bind an antigen when it preferentially recognizes its target antigen in a complex mixture of proteins and/or macromolecules.

The term "surface plasmon resonance", as used herein, refers to an optical phenomenon that allows for the analysis of real-time biospecific interactions by detection of alterations in protein concentrations within a biosensor matrix, for example using the BIAcore system (Pharmacia Biosensor AB, Uppsala, Sweden and Piscataway, NJ). For further descriptions, see Jönsson, U., *et al.* (1993) *Ann. Biol. Clin.* 51:19-26; Jönsson, U., *et al.* (1991) *Biotechniques* 11:620-627; Johnsson, B., *et al.* (1995) *J. Mol. Recognit.* 8:125-131; and Johnnson, B., *et al.* (1991) *Anal. Biochem.* 198:268-277.

The term "K_{On}", as used herein, is intended to refer to the on rate constant for association of an antibody to the antigen to form the antibody/antigen complex as is known in the art.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6615

The term "K_{off}", as used herein, is intended to refer to the off rate constant for dissociation of an antibody from the antibody/antigen complex as is known in the art.

The term "K_d" or "K_D", as used herein, is intended to refer to the dissociation constant of a particular antibody-antigen interaction as is known in the art.

The term "labeled binding protein" as used herein, refers to a protein with a label incorporated that provides for the identification of the binding protein. Preferably, the label is a detectable marker, e.g., incorporation of a radiolabeled amino acid or attachment to a polypeptide of biotinyl moieties that can be detected by marked avidin (e.g., streptavidin containing a fluorescent marker or enzymatic activity that can be detected by optical or colorimetric methods). Examples of labels for polypeptides include, but are not limited to, the following: radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm); fluorescent labels (e.g., FITC, rhodamine, lanthanide phosphors), enzymatic labels (e.g., horseradish peroxidase, luciferase, alkaline phosphatase); chemiluminescent markers; biotinyl groups; predetermined polypeptide epitopes recognized by a secondary reporter (e.g., leucine zipper pair sequences, binding sites for secondary antibodies, metal binding domains, epitope tags); and magnetic agents, such as gadolinium chelates.

The term "antibody conjugate" refers to a binding protein, such as an antibody, chemically linked to a second chemical moiety, such as a therapeutic or cytotoxic agent. The term "agent" is used herein to denote a chemical compound, a mixture of chemical compounds, a biological macromolecule, or an extract made from biological materials. Preferably the therapeutic or cytotoxic agents include, but are not limited to, pertussis toxin, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6616

mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof.

The terms “crystal”, and “crystallized” as used herein, refer to an antibody, or antigen-binding portion thereof, that exists in the form of a crystal. Crystals are one form of the solid state of matter, which is distinct from other forms such as the amorphous solid state or the liquid crystalline state. Crystals are composed of regular, repeating, three-dimensional arrays of atoms, ions, molecules (e.g., proteins such as antibodies), or molecular assemblies (e.g., antigen/antibody complexes). These three-dimensional arrays are arranged according to specific mathematical relationships that are well-understood in the field. The fundamental unit, or building block, that is repeated in a crystal is called the asymmetric unit. Repetition of the asymmetric unit in an arrangement that conforms to a given, well-defined crystallographic symmetry provides the "unit cell" of the crystal. Repetition of the unit cell by regular translations in all three dimensions provides the crystal. *See* Giege, R. and Ducruix, A. Barrett, Crystallization of Nucleic Acids and Proteins, a Practical Approach, 2nd ed., pp. 20 1-16, Oxford University Press, New York, New York, (1999)."

The term "polynucleotide" as referred to herein means a polymeric form of two or more nucleotides, either ribonucleotides or deoxvnucleotides or a modified form of either type of nucleotide. The term includes single and double stranded forms of DNA but preferably is double-stranded DNA.

The term "isolated polynucleotide" as used herein shall mean a polynucleotide (e.g., of genomic, cDNA, or synthetic origin, or some combination thereof) that, by virtue of its origin, is not associated with all or a portion of a polynucleotide with which the "isolated polynucleotide" is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6617

found in nature; is operably linked to a polynucleotide that it is not linked to in nature; or does not occur in nature as part of a larger sequence.

The term "vector", as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a "plasmid", which refers to a circular double stranded DNA loop into which additional DNA segments may be ligated. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (e.g., bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (e.g., non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the invention is intended to include such other forms of expression vectors, such as viral vectors (e.g., replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "operably linked" refers to a juxtaposition wherein the components described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. "Operably linked" sequences include both

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6618

expression control sequences that are contiguous with the gene of interest and expression control sequences that act in *trans* or at a distance to control the gene of interest. The term "expression control sequence" as used herein refers to polynucleotide sequences that are necessary to effect the expression and processing of coding sequences to which they are ligated. Expression control sequences include appropriate transcription initiation, termination, promoter and enhancer sequences; efficient RNA processing signals such as splicing and polyadenylation signals; sequences that stabilize cytoplasmic mRNA; sequences that enhance translation efficiency (i.e., Kozak consensus sequence); sequences that enhance protein stability; and when desired, sequences that enhance protein secretion. The nature of such control sequences differs depending upon the host organism; in prokaryotes, such control sequences generally include promoter, ribosomal binding site, and transcription termination sequence; in eukaryotes, generally, such control sequences include promoters and transcription termination sequence. The term "control sequences" is intended to include components whose presence is essential for expression and processing, and can also include additional components whose presence is advantageous, for example, leader sequences and fusion partner sequences.

"Transformation", as defined herein, refers to any process by which exogenous DNA enters a host cell. Transformation may occur under natural or artificial conditions using various methods well known in the art. Transformation may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method is selected based on the host cell being transformed and may include, but is not limited to, viral infection, electroporation, lipofection, and particle bombardment. Such "transformed" cells include stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6619

as part of the host chromosome. They also include cells that transiently express the inserted DNA or RNA for limited periods of time.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which exogenous DNA has been introduced. It should be understood that such terms are intended to refer not only to the particular subject cell but to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Preferably host cells include prokaryotic and eukaryotic cells selected from any of the Kingdoms of life. Preferred eukaryotic cells include protist, fungal, plant and animal cells. Most preferably host cells include but are not limited to the prokaryotic cell line *E. coli*; mammalian cell lines CHO, HEK 293 and COS; the insect cell line Sf9; and the fungal cell *Saccharomyces cerevisiae*.

Standard techniques may be used for recombinant DNA, oligonucleotide synthesis, and tissue culture and transformation (e.g., electroporation, lipofection). Enzymatic reactions and purification techniques may be performed according to manufacturer's specifications or as commonly accomplished in the art or as described herein. The foregoing techniques and procedures may be generally performed according to conventional methods well known in the art and as described in various general and more specific references that are cited and discussed throughout the present specification. *See e.g.*, Sambrook et al. Molecular Cloning: A Laboratory Manual (2d ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y. (1989)), which is incorporated herein by reference for any purpose.

"Transgenic organism", as known in the art and as used herein, refers to an organism having cells that contain a transgene, wherein the transgene introduced into the organism

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6620

(or an ancestor of the organism) expresses a polypeptide not naturally expressed in the organism. A "transgene" is a DNA construct, which is stably and operably integrated into the genome of a cell from which a transgenic organism develops, directing the expression of an encoded gene product in one or more cell types or tissues of the transgenic organism.

The term "regulate"and "modulate" are used interchangeably, and, as used herein, refers to a change or an alteration in the activity of a molecule of interest (e.g., the biological activity of Aβ(1-42) globulomer). Modulation may be an increase or a decrease in the magnitude of a certain activity or function of the molecule of interest. Exemplary activities and functions of a molecule include, but are not limited to, binding characteristics, enzymatic activity, cell receptor activation, and signal transduction.

Correspondingly, the term "modulator," as used herein, is a compound capable of changing or altering an activity or function of a molecule of interest (e.g., the biological activity of Aβ(1-42) globulomer). For example, a modulator may cause an increase or decrease in the magnitude of a certain activity or function of a molecule compared to the magnitude of the activity or function observed in the absence of the modulator. In certain embodiments, a modulator is an inhibitor, which decreases the magnitude of at least one activity or function of a molecule. Exemplary inhibitors include, but are not limited to, proteins, peptides, antibodies, peptibodies, carbohydrates or small organic molecules. Peptibodies are described, e.g., in International Application Publication No. WO 01/83525.

The term "agonist", as used herein, refers to a modulator that, when contacted with a molecule of interest, causes an increase in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the agonist. Particular agonists of interest may include, but are not limited to, Aβ(1-42) globulomer polypeptides or polypeptides, nucleic

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6621

acids, carbohydrates, or any other molecules that bind to Aβ(1-42) globulomer.

The term "antagonist" or "inhibitor", as used herein, refer to a modulator that, when contacted with a molecule of interest causes a decrease in the magnitude of a certain activity or function of the molecule compared to the magnitude of the activity or function observed in the absence of the antagonist. Particular antagonists of interest include those that block or modulate the biological or immunological activity of Aβ(1-42) globulomer. Antagonists and inhibitors of Aβ(1-42) globulomer may include, but are not limited to, proteins, nucleic acids, carbohydrates, or any other molecules, which bind to Aβ(1-42) globulomer.

As used herein, the term "effective amount" refers to the amount of a therapy which is sufficient to reduce or ameliorate the severity and/or duration of a disorder or one or more symptoms thereof, prevent the advancement of a disorder, cause regression of a disorder, prevent the recurrence, development, onset or progression of one or more symptoms associated with a disorder, detect a disorder, or enhance or improve the prophylactic or therapeutic effect(s) of another therapy (e.g., prophylactic or therapeutic agent).

The term "sample", as used herein, is used in its broadest sense. A “biological sample”, as used herein, includes, but is not limited to, any quantity of a substance from a living thing or formerly living thing. Such living things include, but are not limited to, humans, mice, rats, monkeys, dogs, rabbits and other mammalian or non-mammalian animals. Such substances include, but are not limited to, blood, serum, urine, synovial fluid, cells, organs, tissues (e.g., brain), bone marrow, lymph nodes, cerebrospinal fluid, and spleen.

ANTIBODIES THAT BIND Aß(1-42) GLOBULOMER

One aspect of the present invention provides isolated murine monoclonal antibodies, or antigen-binding portions

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6622

thereof, that bind to Aß(1-42) globulomer with high affinity, a slow off rate and high neutralizing capacity. A second aspect of the invention provides chimeric antibodies that bind Aß(1-42) globulomer. A third aspect of the invention provides CDR grafted antibodies, or antigen-binding portions thereof, that bind Aß(1-42) globulomer. A fourth aspect of the invention provides humanized antibodies, or antigen-binding portions thereof, that bind Aß(1-42) globulomer. Preferably, the antibodies, or portions thereof, are isolated antibodies. Preferably, the antibodies of the invention are neutralizing human anti- Aß(1-42) globulomer antibodies.

A. METHOD OF MAKING ANTI-Aß(1-42) GLOBULOMER ANTIBODIES

Antibodies of the present invention may be made by any of a number of techniques known in the art. Several of these methods are described in detail as follows:

1. ANTI-Aß(1-42) GLOBULOMER MONOCLONAL ANTIBODIES USING HYBRIDOMA TECHNOLOGY

Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including the use of hybridoma, recombinant, and phage display technologies, or a combination thereof. For example, monoclonal antibodies can be produced using hybridoma techniques including those known in the art and taught, for example, in Harlow et al., Antibodies: A Laboratory Manual (Cold Spring Harbor Laboratory Press, 2nd ed. 1988); Hammerling, et al., in: Monoclonal Antibodies and T-Cell Hybridomas 563-681 (Elsevier, N.Y., 1981) (said references incorporated by reference in their entireties). The term "monoclonal antibody" as used herein is not limited to antibodies produced through hybridoma technology. The term "monoclonal antibody" refers to an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced.

Methods for producing and screening for specific antibodies using hybridoma technology are routine and well

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6623

known in the art. In one embodiment, the present invention provides methods of generating monoclonal antibodies as well as antibodies produced by the method comprising culturing a hybridoma cell secreting an antibody of the invention wherein, preferably, the hybridoma is generated by fusing splenocytes isolated from a mouse immunized with an antigen of the invention with myeloma cells and then screening the hybridomas resulting from the fusion for hybridoma clones that secrete an antibody able to bind a polypeptide of the invention. Briefly, mice can be immunized with an Aß(1-42) globulomer antigen. In a preferred embodiment, the antigen is administered with a adjuvant to stimulate the immune response. Such adjuvants include complete or incomplete Freund's adjuvant, RIBI (muramyl dipeptides) or ISCOM (immunostimulating complexes). Such adjuvants may protect the polypeptide from rapid dispersal by sequestering it in a local deposit, or they may contain substances that stimulate the host to secrete factors that are chemotactic for macrophages and other components of the immune system. Preferably, if a polypeptide is being administered, the immunization schedule will involve two or more administrations of the polypeptide, spread out over several weeks.

After immunization of an animal with an Aß(1-42) globulomer antigen, antibodies and/or antibody-producing cells may be obtained from the animal. An anti-Aß(1-42) globulomer antibody-containing serum is obtained from the animal by bleeding or sacrificing the animal. The serum may be used as it is obtained from the animal, an immunoglobulin fraction may be obtained from the serum, or the anti-Aß(1-42) globulomer antibodies may be purified from the serum. Serum or immunoglobulins obtained in this manner are polyclonal, thus having a heterogeneous array of properties.

Once an immune response is detected, e.g., antibodies specific for the antigen Aß(1-42) globulomer are detected in the mouse serum, the mouse spleen is harvested and splenocytes

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6624

isolated. The splenocytes are then fused by well-known techniques to any suitable myeloma cells, for example cells from cell line SP20 available from the American Type Culture Collection (Manassas, VA). Hybridomas are selected and cloned by limited dilution. The hybridoma clones are then assayed by methods known in the art for cells that secrete antibodies capable of binding Aß(1-42) globulomer. Ascites fluid, which generally contains high levels of antibodies, can be generated by immunizing mice with positive hybridoma clones.

In another embodiment, antibody-producing immortalized hybridomas may be prepared from the immunized animal. After immunization, the animal is sacrificed and the splenic B cells are fused to immortalized myeloma cells as is well known in the art. *See, e.g.*, Harlow and Lane, *supra*. In a preferred embodiment, the myeloma cells do not secrete immunoglobulin polypeptides (a non-secretory cell line). After fusion and antibiotic selection, the hybridomas are screened using Aß(1-42) globulomer, or a portion thereof, or a cell expressing Aß(1-42) globulomer. In a preferred embodiment, the initial screening is performed using an enzyme-linked immunoassay (ELISA) or a radioimmunoassay (RIA), preferably an ELISA. An example of ELISA screening is provided in International Application Publication No. WO 00/37504, herein incorporated by reference.

Anti-Aß(1-42) globulomer antibody-producing hybridomas are selected, cloned and further screened for desirable characteristics, including robust hybridoma growth, high antibody production and desirable antibody characteristics, as discussed further below. Hybridomas may be cultured and expanded *in vivo* in syngeneic animals, in animals that lack an immune system, e.g., nude mice, or in cell culture *in vitro*. Methods of selecting, cloning and expanding hybridomas are well known to those of ordinary skill in the art.

In a preferred embodiment, the hybridomas are mouse hybridomas, as described above. In another preferred embodiment, the hybridomas are produced in a non-human, non-

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6625

mouse species such as rats, sheep, pigs, goats, cattle or horses. In another embodiment, the hybridomas are human hybridomas, in which a human non-secretory myeloma is fused with a human cell expressing an anti-Aß(1-42) globulomer antibody.

Antibody fragments that recognize specific epitopes may be generated by known techniques. For example, Fab and F(ab')2 fragments of the invention may be produced by proteolytic cleavage of immunoglobulin molecules, using enzymes such as papain (to produce Fab fragments) or pepsin (to produce F(ab')2 fragments). F(ab')2 fragments contain the variable region, the light chain constant region and the CHI domain of the heavy chain.

2. ANTI-Aß(1-42) GLOBULOMER MONOCLONAL ANTIBODIES USING SLAM

In another aspect of the invention, recombinant antibodies are generated from single, isolated lymphocytes using a procedure referred to in the art as the selected lymphocyte antibody method (SLAM), as described in U.S. Patent No. 5,627,052, International Application Publication No. WO 92/02551 and Babcock, J.S. *et al.* (1996) *Proc. Natl. Acad. Sci. USA* 93:7843-7848. In this method, single cells secreting antibodies of interest, *e.g.*, lymphocytes derived from any one of the immunized animals described in Section 1, are screened using an antigen-specific hemolytic plaque assay, wherein the antigen Aß(1-42) globulomer, a subunit of Aß(1-42) globulomer, or a fragment thereof, is coupled to sheep red blood cells using a linker, such as biotin, and used to identify single cells that secrete antibodies with specificity for Aß(1-42) globulomer. Following identification of antibody-secreting cells of interest, heavy- and light-chain variable region cDNAs are rescued from the cells by reverse transcriptase-PCR and these variable regions can then be expressed, in the context of appropriate immunoglobulin constant regions (*e.g.*, human constant regions), in mammalian host cells, such as COS

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6626

or CHO cells. The host cells transfected with the amplified immunoglobulin sequences, derived from *in vivo* selected lymphocytes, can then undergo further analysis and selection *in vitro*, for example by panning the transfected cells to isolate cells expressing antibodies to Aß(1-42) globulomer. The amplified immunoglobulin sequences further can be manipulated *in vitro*, such as by *in vitro* affinity maturation methods such as those described in International Application Publication No. WO 97/29131 and International Application Publication No. WO 00/56772.

3. ANTI-Aß(1-42) GLOBULOMER MONOCLONAL ANTIBODIES USING TRANSGENIC ANIMALS

In another embodiment of the instant invention, antibodies are produced by immunizing a non-human animal comprising some, or all, of the human immunoglobulin locus with an Aß(1-42) globulomer antigen. In a preferred embodiment, the non-human animal is a XENOMOUSE transgenic mouse, an engineered mouse strain that comprises large fragments of the human immunoglobulin loci and is deficient in mouse antibody production. *See, e.g.,* Green et al. *Nature Genetics* 7:13-21 (1994) and United States Patent Nos. 5,916,771, 5,939,598, 5,985,615, 5,998,209, 6,075,181, 6,091,001, 6,114,598 and 6,130,364. *See also* Internation Appln. Publication No. WO 91/10741, published July 25,1991, WO 94/02602, published February 3, 1994, WO 96/34096 and WO 96/33735, both published October 31, 1996, WO 98/16654, published April 23, 1998, WO 98/24893, published June 11, 1998, WO 98/50433, published November 12, 1998, WO 99/45031, published September 10, 1999, WO 99/53049, published October 21, 1999, WO 00/ 09560, published February 24, 2000 and WO 00/037504, published June 29, 2000. The XENOMOUSE transgenic mouse produces an adult-like human repertoire of fully human antibodies and generates antigen-specific human Mabs. The XENOMOUSE transgenic mouse contains approximately 80% of the human antibody repertoire through introduction of megabase

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6627

sized, germline configuration YAC fragments of the human heavy chain loci and x light chain loci. *See* Mendez et al., *Nature Genetics* 15:146-156 (1997), Green and Jakobovits *J. Exp. Med.* 188:483-495 (1998), the disclosures of which are hereby incorporated by reference.

4. ANTI-Aß(1-42) GLOBULOMER MONOCLONAL ANTIBODIES USING RECOMBINANT ANTIBODY LIBRARIES

In vitro methods also can be used to make the antibodies of the invention, wherein an antibody library is screened to identify an antibody having the desired binding specificity. Methods for such screening of recombinant antibody libraries are well known in the art and include methods described in, for example, Ladner *et al.*, U.S. Patent No. 5,223,409; Kang *et al.*, International Appln. Publication No. WO 92/18619; Dower *et al.*, International Appln. Publication No. WO 91/17271; Winter *et al.*, International Appln. Publication No. WO 92/20791; Markland *et al.*, International Appln. Publication No. WO 92/15679; Breitling *et al.*, International Appln. Publication No. WO 93/01288; McCafferty *et al.*, PCT Publication No. WO 92/01047; Garrard *et al.*, International Appln. Publication No. WO 92/09690; Fuchs *et al.* (1991), *Bio/Technology* 9:1370-1372; Hay *et al.*, (1992) *Hum Antibod Hybridomas* 3:81-85; Huse *et al.* (1989), *Science* 246:1275-1281; McCafferty *et al.*, *Nature* (1990) 348:552-554; Griffiths *et al.* (1993) *EMBO J* 12:725-734; Hawkins *et al.*, (1992) *J Mol Biol* 226:889-896; Clackson *et al.*, (1991) *Nature* 352:624-628; Gram *et al.*, (1992) *PNAS* 89:3576-3580; Garrad *et al.* (1991) *Bio/Technology* 9:1373-1377; Hoogenboom *et al.* (1991), *Nuc Acid Res* 19:4133-4137; and Barbas *et al.* (1991), *PNAS* 88:7978-7982, U.S. Patent Application Publication No. 20030186374, and International Application Publication No. WO 97/29131, the contents of each of which are incorporated herein by reference.

The recombinant antibody library may be from a subject immunized with Aß(1-42) globulomer, or a portion of Aß(1-42)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6628

globulomer. Alternatively, the recombinant antibody library may be from a naïve subject, i.e., one who has not been immunized with Aß(1-42) globulomer, such as a human antibody library from a human subject who has not been immunized with human Aß(1-42) globulomer. Antibodies of the invention are selected by screening the recombinant antibody library with the peptide comprising human Aß(1-42) globulomer to thereby select those antibodies that recognize Aß(1-42) globulomer. Methods for conducting such screening and selection are well known in the art, such as described in the references in the preceding paragraph. To select antibodies of the invention having particular binding affinities for Aß(1-42) globulomer, such as those that dissociate from human Aß(1-42) globulomer with a particular k_{off} rate constant, the art-known method of surface plasmon resonance can be used to select antibodies having the desired k_{off} rate constant. To select antibodies of the invention having a particular neutralizing activity for Aß(1-42) globulomer, such as those with a particular IC_{50}, standard methods known in the art for assessing the inhibition of human Aß(1-42) globulomer activity may be used.

In one aspect, the invention pertains to an isolated antibody, or an antigen-binding portion thereof, that binds human Aß(1-42) globulomer. Preferably, the antibody is a neutralizing antibody. In various embodiments, the antibody is a recombinant antibody or a monoclonal antibody.

For example, the antibodies of the present invention can also be generated using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of phage particles that carry the polynucleotide sequences encoding them. In a particular, such phage can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library (e.g., human or murine). Phage expressing an antigen binding domain that binds the antigen of interest can be selected or identified with antigen, e.g., using labeled antigen or antigen bound or captured to a solid surface or

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6629

bead. Phage used in these methods are typically filamentous phage including fd and M13 binding domains expressed from phage with Fab, Fv or disulfide stabilized Fv antibody domains recombinantly fused to either the phage gene III or gene VIII protein. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Brinkman et al., *J. Immunol. Methods* 182:41-50 (1995); Ames et al., *J. Immunol. Methods* 184:177-186 (1995); Kettleborough et al., *Eur. J. Immunol.* 24:952-958 (1994); Persic et al., *Gene* 187 9-18 (1997); Burton et al., *Advances in Immunology* 57:191-280 (1994); International Application No. PCT/GB91/01134; International Appln. Publication Nos. WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780, 225; 5,658,727; 5,733,743 and 5,969,108, each of which is incorporated herein by reference in its entirety.

As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole antibodies including human antibodies or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below. For example, techniques to recombinantly produce Fab, Fab' and F(ab')2 fragments can also be employed using methods known in the art such as those disclosed in International Application Publ. No. WO 92/22324; Mullinax et al., *BioTechniques* 12(6):864-869 (1992); and Sawai et al., *AJRI* 34:26-34 (1995); and Better et al., *Science* 240:1041-1043 (1988) (said references incorporated by reference in their entireties). Examples of techniques which can be used to produce single-chain Fvs and antibodies include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston et al., *Methods in Enzymology* 203:46-88 (1991); Shu et

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6630

al., *PNAS* 90:7995-7999 (1993); and Skerra et al., *Science* 240:1038-1040 (1988).

Alternative to screening of recombinant antibody libraries by phage display, other methodologies known in the art for screening large combinatorial libraries can be applied to the identification of dual specificity antibodies of the invention. One type of alternative expression system is one in which the recombinant antibody library is expressed as RNA-protein fusions, as described in International Appln. Publication No. WO 98/31700 by Szostak and Roberts, and in Roberts, R.W. and Szostak, J.W. (1997) *Proc. Natl. Acad. Sci. USA* 94:12297-12302. In this system, a covalent fusion is created between an mRNA and the peptide or protein that it encodes by *in vitro* translation of synthetic mRNAs that carry puromycin, a peptidyl acceptor antibiotic, at their 3' end. Thus, a specific mRNA can be enriched from a complex mixture of mRNAs (*e.g.*, a combinatorial library) based on the properties of the encoded peptide or protein, *e.g.*, antibody, or portion thereof, such as binding of the antibody, or portion thereof, to the dual specificity antigen. Nucleic acid sequences encoding antibodies, or portions thereof, recovered from screening of such libraries can be expressed by recombinant means as described above (*e.g.*, in mammalian host cells) and, moreover, can be subjected to further affinity maturation by either additional rounds of screening of mRNA-peptide fusions in which mutations have been introduced into the originally selected sequence(s), or by other methods for affinity maturation *in vitro* of recombinant antibodies, as described above.

In another approach the antibodies of the present invention can also be generated using yeast display methods known in the art. In yeast display methods, genetic methods are used to tether antibody domains to the yeast cell wall and display them on the surface of yeast. In particular, such yeast can be utilized to display antigen-binding domains expressed from a repertoire or combinatorial antibody library

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6631

(e.g., human or murine). Examples of yeast display methods that can be used to make the antibodies of the present invention include those disclosed Wittrup et al., U.S. Patent No. 6,699,658 incorporated herein by reference.

B. PRODUCTION OF RECOMBINANT Aß(1-42) GLOBULOMER ANTIBODIES

As noted above, antibodies of the present invention may be produced by any of a number of techniques known in the art. For example, expression from host cells, wherein expression vector(s) encoding the heavy and light chains is (are) transfected into a host cell by standard techniques. The various forms of the term "transfection" are intended to encompass a wide variety of techniques commonly used for the introduction of exogenous DNA into a prokaryotic or eukaryotic host cell, *e.g.*, electroporation, calcium-phosphate precipitation, DEAE-dextran transfection and the like. Although it is possible to express the antibodies of the invention in either prokaryotic or eukaryotic host cells, expression of antibodies in eukaryotic cells is preferable, and most preferable in mammalian host cells, because such eukaryotic cells (and in particular mammalian cells) are more likely than prokaryotic cells to assemble and secrete a properly folded and immunologically active antibody.

Preferred mammalian host cells for expressing the recombinant antibodies of the invention include Chinese Hamster Ovary (CHO cells) (including dhfr- CHO cells, described in Urlaub and Chasin, (1980) *Proc. Natl. Acad. Sci. USA* 77:4216-4220, used with a DHFR selectable marker, *e.g.*, as described in R.J. Kaufman and P.A. Sharp (1982) *Mol. Biol.* 159:601-621), NS0 myeloma cells, COS cells and SP2 cells. When recombinant expression vectors encoding antibody genes are introduced into mammalian host cells, the antibodies are produced by culturing the host cells for a period of time sufficient to allow for expression of the antibody in the host cells or, more preferably, secretion of the antibody into the culture medium in which the host cells are grown. Antibodies

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6632

can be recovered from the culture medium using standard protein purification methods.

Host cells can also be used to produce functional antibody fragments, such as Fab fragments or scFv molecules. It will be understood that variations on the above procedure are within the scope of the present invention. For example, it may be desirable to transfect a host cell with DNA encoding functional fragments of either the light chain and/or the heavy chain of an antibody of this invention. Recombinant DNA technology may also be used to remove some, or all, of the DNA encoding either or both of the light and heavy chains that is not necessary for binding to the antigens of interest. The molecules expressed from such truncated DNA molecules are also encompassed by the antibodies of the invention. In addition, bifunctional antibodies may be produced in which one heavy and one light chain are an antibody of the invention and the other heavy and light chain are specific for an antigen other than the antigens of interest by crosslinking an antibody of the invention to a second antibody by standard chemical crosslinking methods.

In a preferred system for recombinant expression of an antibody, or antigen-binding portion thereof, of the invention, a recombinant expression vector encoding both the antibody heavy chain and the antibody light chain is introduced into dhfr- CHO cells by calcium phosphate-mediated transfection. Within the recombinant expression vector, the antibody heavy and light chain genes are each operatively linked to CMV enhancer/AdMLP promoter regulatory elements to drive high levels of transcription of the genes. The recombinant expression vector also carries a DHFR gene, which allows for selection of CHO cells that have been transfected with the vector using methotrexate selection/amplification. The selected transformant host cells are cultured to allow for expression of the antibody heavy and light chains and intact antibody is recovered from the culture medium. Standard molecular biology techniques are used to prepare the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6633

recombinant expression vector, transfect the host cells, select for transformants, culture the host cells and recover the antibody from the culture medium. Still further the invention provides a method of synthesizing a recombinant antibody of the invention by culturing a host cell of the invention in a suitable culture medium until a recombinant antibody of the invention is synthesized. The method can further comprise isolating the recombinant antibody from the culture medium.

1. ANTI-Aß(1-42) GLOBULOMER ANTIBODIES

The isolated anti-Aß(1-42) globulomer antibody CDR sequences described herein (see Table 4) establish a novel family of Aß(1-42) globulomer binding proteins, isolated in accordance with this invention, and comprising polypeptides that include the CDR sequences listed above. To generate and to select CDRs of the invention having preferred Aß(1-42) globulomer binding and/or neutralizing activity with respect to Aß(1-42) globulomer, standard methods known in the art for generating binding proteins of the present invention and assessing the Aß(1-42) globulomer binding and/or neutralizing characteristics of those binding protein may be used, including but not limited to those specifically described herein.

2. ANTI-Aß(1-42) GLOBULOMER CHIMERIC ANTIBODIES

A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal antibody and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art and discussed in detail in Example 2.1. See e.g., Morrison, *Science* 229:1202 (1985); Oi et al., BioTechniques 4:214 (1986); Gillies et al., (1989) J. *Immunol. Methods* 125:191-202; U.S. Pat. Nos. 5,807,715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6634

entireties. In addition, techniques developed for the production of "chimeric antibodies" (Morrison et al., 1984, *Proc. Natl. Acad. Sci.* 81:851-855; Neuberger et al., 1984, *Nature* 312:604-608; Takeda et al., 1985, *Nature* 314:452-454 which are incorporated herein by reference in their entireties) by splicing genes from a mouse antibody molecule of appropriate antigen specificity together with genes from a human antibody molecule of appropriate biological activity can be used.

In one embodiment, the chimeric antibodies of the invention are produced by replacing the heavy chain constant region of the murine monoclonal anti-human Aß(1-42) globulomer antibodies described above with a human IgG1 constant region. In a specific embodiment, the chimeric antibody of the invention comprises a heavy chain variable region (V_H) comprising the amino acid sequence of SEQ ID NO:1 and a light chain variable region (V_L) comprising the amino acid sequence of SEQ ID NO:2.

3. ANTI-Aß(1-42) GLOBULOMER CDR GRAFTED ANTIBODIES

CDR-grafted antibodies of the invention comprise heavy and light chain variable region sequences from a human antibody wherein one or more of the CDR regions of V_H and/or V_L are replaced with CDR sequences of the murine antibodies of the invention. A framework sequence from any human antibody may serve as the template for CDR grafting. However, straight chain replacement onto such a framework often leads to some loss of binding affinity to the antigen. The more homologous a human antibody is to the original murine antibody, the less likely the possibility that combining the murine CDRs with the human framework will introduce distortions in the CDRs that could reduce affinity. Therefore, it is preferable that the human variable framework that is chosen to replace the murine variable framework apart from the CDRs have at least a 65% sequence identity with the murine antibody variable region

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6635

framework. It is more preferable that the human and murine variable regions apart from the CDRs have at least 70% sequence identify. It is even more preferable that the human and murine variable regions apart from the CDRs have at least 75% sequence identity. It is most preferable that the human and murine variable regions apart from the CDRs have at least 80% sequence identity. Methods for producing chimeric antibodies are known in the art and discussed in detail in Example 2.2. (*See also* EP 239,400; Intern. Appln. Publication No. WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994)), and chain shuffling (U.S. Pat. No. 5,565,352).

4. ANTI-Aß(1-42) GLOBULOMER HUMANIZED ANTIBODIES

Table 4 below includes a list of amino acid sequences of VH and VL regions of preferred anti-Aß(1-42) humanized globulomer antibodies of the invention as well as the CDRs contained therein.

Table 4: LIST OF AMINO ACID SEQUENCES OF VH AND VL REGIONS

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
1	**VH 8F5 (hum8)**		EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLELVASINSNGGSTYYPDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCASGDYWGQGTLVTVSS **(SEQ ID NO.:1)**
	VH 8F5 **(hum8)** CDR-H1	Residues 31-35 of SEQ ID NO.:1	**SYGMS (SEQ ID NO:11)**
	VH 8F5 **(hum8)** CDR-H2	Residues 50-66 of SEQ ID NO.:1	**SINSNGGSTYYPDSVKG (SEQ ID NO:12)**
	VH 8F5 **(hum8)**	Residues 98-108 of SEQ ID	**SGDY (SEQ ID NO:13)**

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6636

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**1234567 89**0**
	CDR-H3	NO.:1	
2	**VL 8F5 (hum8)**		DIVMTQSPLSLPVTPGEPASISCRSSQSL VYSNGDTYLHWYLQKPGQSPKLLIYKV SNRFSGVPDRFSGSGSGTDFTLKISRVE AEDVGVYYCSQSTHVPWTFGGGTKVEI KR
	VL 8F5 **(hum8)** CDR-L1	Residues 24-39 of SEQ ID NO.:2	**RSSQSLVYSNGDTYLH (SEQ ID NO:14)**
	VL 8F5 **(hum8)** CDR-L2	Residues 55-61 of SEQ ID NO.:2	**KVSNRFS (SEQ ID NO:15)**
	VL 8F5 **(hum8)** CDR-L3	Residues 94-102 of SEQ ID NO.:2	**SQSTHVPWT (SEQ ID NO:16)**

Humanized antibodies are antibody molecules from non-human species antibody that bind the desired antigen having one or more complementarity determining regions (CDRs) from the non-human species and framework regions from a human immunoglobulin molecule. Known human Ig sequences are disclosed, e.g., www.ncbi.nlm.nih.gov/entrez- /query.fcgi; www.atcc.org/phage/hdb.html; www.sciquest.com/; www.abcam.com/; www.antibodyresource.com/onlinecomp.html; www.public.iastate.edu/.about.pedro/research_tools.html; www.mgen.uni-heidelberg.de/SD/IT/IT.html; www.whfreeman.com/immunology/CH- 05/kuby05.htm; www.library.thinkquest.org/12429/Immune/Antibody.html; www.hhmi.org/grants/lectures/1996/vlab/; www.path.cam.ac.uk/.about.mrc7/m- ikeimages.html; www.antibodyresource.com/; mcb.harvard.edu/BioLinks/Immunology.html.www.immunologylink.com/; pathbox.wustl.edu/.about.hcenter/index.- html; www.biotech.ufl.edu/.about.hcl/; www.pebio.com/pa/340913/340913.html- ; www.nal.usda.gov/awic/pubs/antibody/; www.m.ehime-u.acjp/.about.yasuhito- /Elisa.html; www.biodesign.com/table.asp; www.icnet.uk/axp/facs/davies/lin-

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6637

ks.html; www.biotech.ufl.edu/.about.fccl/protocol.html; www.isac-net.org/sites_geo.html; aximtl.imt.uni-marburg.de/.about.rek/AEP- Start.html; baserv.uci.kun.nl/.about.jraats/linksl.html; www.recab.uni-hd.de/immuno.bme.nwu.edu/; www.mrc-cpe.cam.ac.uk/imt-doc/public/INTRO.html; www.ibt.unam.mx/vir/V_mice.html; imgt.cnusc.fr:8104/; www.biochem.ucl.ac.uk/.about.martin/abs/index.html; antibody.bath.ac.uk/; abgen.cvm.tamu.edu/lab/wwwabgen.html; www.unizh.ch/.about.honegger/AHOsem- inar/Slide01.html; www.cryst.bbk.ac.uk/.about.ubcg07s/; www.nimr.mrc.ac.uk/CC/ccaewg/ccaewg.htm; www.path.cam.ac.uk/.about.mrc7/h- umanisation/TAHHP.html; www.ibt.unam.mx/vir/structure/stat_aim.html; www.biosci.missouri.edu/smithgp/index.html; www.cryst.bioc.cam.ac.uk/.abo- ut.fmolina/Webpages/Pept/spottech.html; www.jerini.de/fr roducts.htm; www.patents.ibm.com/ibm.html.Kabat et al., Sequences of Proteins of Immunological Interest, U.S. Dept. Health (1983), each entirely incorporated herein by reference. Such imported sequences can be used to reduce immunogenicity or reduce, enhance or modify binding, affinity, on-rate, off-rate, avidity, specificity, half-life, or any other suitable characteristic, as known in the art.

Framework residues in the human framework regions may be substituted with the corresponding residue from the CDR donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding and sequence comparison to identify unusual framework residues at particular positions. (*See, e.g.*, Queen et al., U.S. Pat. No. 5,585,089; Riechmann et al., *Nature* 332:323 (1988), which are incorporated herein by reference in their entireties.) Three-dimensional immunoglobulin models are commonly available and are familiar

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6638

to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding. Antibodies can be humanized using a variety of techniques known in the art, such as but not limited to those described in Jones et al., Nature 321:522 (1986); Verhoeyen et al., *Science* 239:1534 (1988)), Sims et al., *J. Immunol.* 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987), Carter et al., *Proc. Natl. Acad. Sci. U.S.A.* 89:4285 (1992); Presta et al., J. Immunol. 151:2623 (1993), Padlan, *Molecular Immunology* 28(4/5):489-498 (1991); Studnicka et al., *Protein Engineering* 7(6):805-814 (1994); Roguska et al., *PNAS* 91:969-973 (1994); International Appln. Publication No. WO 91/09967, PCT/: US98/16280, US96/18978, US91/09630, US91/05939, US94/01234, GB89/01334, GB91/01134, GB92/01755; WO90/14443, WO90/14424, WO90/14430, EP 229246, EP 592,106; EP 519,596, EP 239,400, U.S. Pat. Nos. 5,565,332, 5,723,323, 5,976,862, 5,824,514, 5,817,483, 5814476, 5763192, 5723323, 5,766886, 5,714,352, 6,204,023, 6,180,370, 5,693,762, 5,530,101, 5,585,089, 5,225,539; 4,816,567, each entirely incorporated herein by reference, included references cited therein.

C. Production of Antibodies and Antibody-Producing Cell Lines

As noted above, preferably, anti-Aß(1-42) globulomer antibodies of the present invention exhibit a high capacity to reduce or to neutralize Aß(1-42) globulomer activity, e.g., as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6639

assessed by any one of several *in vitro* and *in vivo* assays known in the art (e.g., see Examples below).

In certain embodiments, the antibody comprises a heavy chain constant region, such as an IgG1, IgG2, IgG3, IgG4, IgA, IgE, IgM or IgD constant region. Preferably, the heavy chain constant region is an IgG1 heavy chain constant region or an IgG4 heavy chain constant region. Furthermore, the antibody can comprise a light chain constant region, either a kappa light chain constant region or a lambda light chain constant region. Preferably, the antibody comprises a kappa light chain constant region. Alternatively, the antibody portion can be, for example, a Fab fragment or a single chain Fv fragment.

Replacements of amino acid residues in the Fc portion to alter antibody effector function are known in the art (Winter *et al.*, U.S. Patent Nos. 5,648,260 and 5,624,821). The Fc portion of an antibody mediates several important effector functions, for example, cytokine induction, ADCC, phagocytosis, complement dependent cytotoxicity (CDC) and half-life/ clearance rate of antibody and antigen-antibody complexes. In some cases these effector functions are desirable for therapeutic antibody but in other cases might be unnecessary or even deleterious, depending on the therapeutic objectives. Certain human IgG isotypes, particularly IgG1 and IgG3, mediate ADCC and CDC via binding to FcγRs and complement C1q, respectively. Neonatal Fc receptors (FcRn) are the critical components determining the circulating half-life of antibodies. In still another embodiment, at least one amino acid residue is replaced in the constant region of the antibody, for example the Fc region of the antibody, such that effector functions of the antibody are altered.

One embodiment provides a labeled binding protein wherein an antibody or antibody portion of the invention is derivatized or linked to another functional molecule (e.g., another peptide or protein). For example, a labeled binding protein of the invention can be derived by functionally

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6640

linking an antibody or antibody portion of the invention (by chemical coupling, genetic fusion, noncovalent association or otherwise) to one or more other molecular entities, such as another antibody (e.g., a bispecific antibody or a diabody), a detectable agent, a cytotoxic agent, a pharmaceutical agent, and/or a protein or peptide that can mediate associate of the antibody or antibody portion with another molecule (such as a streptavidin core region or a polyhistidine tag).

Useful detectable agents with which an antibody or antibody portion of the invention may be derivatized include fluorescent compounds. Exemplary fluorescent detectable agents include fluorescein, fluorescein isothiocyanate, rhodamine, 5-dimethylamine-1-napthalenesulfonyl chloride, phycoerythrin and the like. An antibody may also be derivatized with detectable enzymes, such as alkaline phosphatase, horseradish peroxidase, glucose oxidase and the like. When an antibody is derivatized with a detectable enzyme, it is detected by adding additional reagents that the enzyme uses to produce a detectable reaction product. For example, when the detectable agent horseradish peroxidase is present, the addition of hydrogen peroxide and diaminobenzidine leads to a colored reaction product, which is detectable. An antibody may also be derivatized with biotin, and detected through indirect measurement of avidin or streptavidin binding.

Another embodiment of the invention provides a crystallized binding protein. Preferably, the invention relates to crystals of whole anti-Aß(1-42) globulomer antibodies and fragments thereof as disclosed herein, and formulations and compositions comprising such crystals. In one embodiment the crystallized binding protein has a greater half-life in vivo than the soluble counterpart of the binding protein. In another embodiment, the binding protein retains biological activity after crystallization.

Crystallized binding protein of the invention may be produced according methods known in the art and as disclosed

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6641

in International Appln. Publication No. WO 02/072636, incorporated herein by reference.

Another embodiment of the invention provides a glycosylated binding protein wherein the antibody or antigen-binding portion thereof comprises one or more carbohydrate residues. Nascent *in vivo* protein production may undergo further processing, known as post-translational modification. In particular, sugar (glycosyl) residues may be added enzymatically, a process known as glycosylation. The resulting proteins bearing covalently linked oligosaccharide side chains are known as glycosylated proteins or glycoproteins. Antibodies are glycoproteins with one or more carbohydrate residues in the Fc domain, as well as the variable domain. Carbohydrate residues in the Fc domain have important effect on the effector function of the Fc domain, with minimal effect on antigen binding or half-life of the antibody (R. Jefferis, *Biotechnol. Prog.* 21 (2005), pp. 11-16). In contrast, glycosylation of the variable domain may have an effect on the antigen binding activity of the antibody. Glycosylation in the variable domain may have a negative effect on antibody binding affinity, likely due to steric hindrance (Co, M.S., et al., *Mol. Immunol.* (1993) 30:1361-1367), or result in increased affinity for the antigen (Wallick, S.C., et al., *Exp. Med.* (1988) 168:1099-1109; Wright, A., et al., *EMBO J.* (1991) 10:2717 2723).

One aspect of the present invention is directed to generating glycosylation site mutants in which the O- or N-linked glycosylation site of the binding protein has been mutated. One skilled in the art can generate such mutants using standard well-known technologies. The creation of glycosylation site mutants that retain the biological activity but have increased or decreased binding activity are another object of the present invention.

In still another embodiment, the glycosylation of the antibody or antigen-binding portion of the invention is modified. For example, an aglycoslated antibody can be made

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6642

(i.e., the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for antigen. Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in further detail in International Appln. Publication No. WO 03/016466A2, and U.S. Pat. Nos. 5,714,350 and 6,350,861, each of which is incorporated herein by reference in its entirety.

Additionally or alternatively, a modified antibody of the invention can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. *See*, for example, Shields, R. L. et al. (2002) *J. Biol. Chem.* 277:26733-26740; Umana et al. (1999) *Nat. Biotech.* 17:176-1, as well as, European Patent No: EP 1,176,195; International Appln. Publication Nos. WO 03/035835 and WO 99/5434280, each of which is incorporated herein by reference in its entirety.

Protein glycosylation depends on the amino acid sequence of the protein of interest, as well as the host cell in which the protein is expressed. Different organisms may produce different glycosylation enzymes (e.g., glycosyltransferases and glycosidases), and have different substrates (nucleotide

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6643

sugars) available. Due to such factors, protein glycosylation pattern, and composition of glycosyl residues, may differ depending on the host system in which the particular protein is expressed. Glycosyl residues useful in the invention may include, but are not limited to, glucose, galactose, mannose, fucose, n-acetylglucosamine and sialic acid. Preferably the glycosylated binding protein comprises glycosyl residues such that the glycosylation pattern is human.

It is known to those skilled in the art that differing protein glycosylation may result in differing protein characteristics. For instance, the efficacy of a therapeutic protein produced in a microorganism host, such as yeast, and glycosylated utilizing the yeast endogenous pathway may be reduced compared to that of the same protein expressed in a mammalian cell, such as a CHO cell line. Such glycoproteins may also be immunogenic in humans and show reduced half-life *in vivo* after administration. Specific receptors in humans and other animals may recognize specific glycosyl residues and promote the rapid clearance of the protein from the bloodstream. Other adverse effects may include changes in protein folding, solubility, susceptibility to proteases, trafficking, transport, compartmentalization, secretion, recognition by other proteins or factors, antigenicity, or allergenicity. Accordingly, a practitioner may prefer a therapeutic protein with a specific composition and pattern of glycosylation, for example glycosylation composition and pattern identical, or at least similar, to that produced in human cells or in the species-specific cells of the intended subject animal.

Expressing glycosylated proteins different from that of a host cell may be achieved by genetically modifying the host cell to express heterologous glycosylation enzymes. Using techniques known in the art a practitioner may generate antibodies or antigen-binding portions thereof exhibiting human protein glycosylation. For example, yeast strains have been genetically modified to express non-naturally occurring

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6644

glycosylation enzymes such that glycosylated proteins (glycoproteins) produced in these yeast strains exhibit protein glycosylation identical to that of animal cells, especially human cells (U.S Patent Application Publication Nos. 20040018590 and 20020137134 and International Appln. Publication No. WO 05/100584 A2).

The term "multivalent binding protein" is used in this specification to denote a binding protein comprising two or more antigen binding sites. The multivalent binding protein is preferably engineered to have the three or more antigen binding sites, and is generally not a naturally occurring antibody. The term "multispecific binding protein" refers to a binding protein capable of binding two or more related or unrelated targets. Dual variable domain (DVD) binding proteins as used herein, are binding proteins that comprise two or more antigen binding sites and are tetravalent or multivalent binding proteins. Such DVDs may be monospecific, i.e, capable of binding one antigen or multispecific, i.e., capable of binding two or more antigens. DVD binding proteins comprising two heavy chain DVD polypeptides and two light chain DVD polypeptides are refered to a DVD Ig. Each half of a DVD Ig comprises a heavy chain DVD polypeptide, and a light chain DVD polypeptide, and two antigen binding sites. Each binding site comprises a heavy chain variable domain and a light chain variable domain with a total of 6 CDRs involved in antigen binding per antigen binding site. DVD binding proteins and methods of making DVD binding proteins are disclosed in U.S. Patent Application No. 11/507,050 and incorporated herein by reference.

One aspect of the invention pertains to a DVD binding protein comprising binding proteins capable of binding to Aß(1-42) globulomer. Preferably, the DVD binding protein is capable of binding Aß(1-42) globulomer and a second target.

In addition to the binding proteins, the present invention is also directed to an anti-idiotypic (anti-Id) antibody specific for such binding proteins of the invention.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6645

An anti-Id antibody is an antibody, which recognizes unique determinants generally associated with the antigen-binding region of another antibody. The anti-Id can be prepared by immunizing an animal with the binding protein or a CDR containing region thereof. The immunized animal will recognize, and respond to the idiotypic determinants of the immunizing antibody and produce an anti-Id antibody. The anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody.

Further, it will be appreciated by one skilled in the art that a protein of interest may be expressed using a library of host cells genetically engineered to express various glycosylation enzymes, such that member host cells of the library produce the protein of interest with variant glycosylation patterns. A practitioner may then select and isolate the protein of interest with particular novel glycosylation patterns. Preferably, the protein having a particularly selected novel glycosylation pattern exhibits improved or altered biological properties.

D. Uses of Anti-Aß(1-42) Antibodies

Given their ability to bind to Aß(1-42) globulomer, the anti-Aß(1-42) globulomer antibodies, or portions thereof, of the invention can be used to detect Aß(1-42) globulomer (*e.g.*, in a biological sample such as serum, whole blood, CSF, brain tissue or plasma), using a conventional immunoassay, such as an enzyme linked immunosorbent assays (ELISA), an radioimmunoassay (RIA) or tissue immunohistochemistry. The invention therefore provides a method for detecting Aß(1-42) globulomer in a biological sample comprising contacting a biological sample with an antibody, or antibody portion, of the invention and detecting either the antibody (or antibody portion) bound to Aß(1-42) globulomer or unbound antibody (or antibody portion), to thereby detect Aß(1-42) globulomer in the biological sample. The antibody is directly or indirectly labeled with a detectable substance to facilitate detection of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6646

the bound or unbound antibody. Suitable detectable substances include various enzymes, prosthetic groups, fluorescent materials, luminescent materials and radioactive materials. Examples of suitable enzymes include horseradish peroxidase, alkaline phosphatase, β-galactosidase, or acetylcholinesterase; examples of suitable prosthetic group complexes include streptavidin/biotin and avidin/biotin; examples of suitable fluorescent materials include umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; an example of a luminescent material includes luminol; and examples of suitable radioactive material include ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, or ^{153}Sm.

Alternative to labeling the antibody, Aß(1-42) globulomer can be assayed in biological fluids by a competition immunoassay utilizing recombinant Aß(1-42) globulomer standards labeled with a detectable substance and an unlabeled anti-Aß(1-42) globulomer antibody. In this assay, the biological sample, the labeled recombinant Aß(1-42) globulomer standards and the anti-Aß(1-42) globulomer antibody are combined, and the amount of labeled recombinant Aß(1-42) globulomer standard bound to the unlabeled antibody is determined. The amount of Aß(1-42) globulomer in the biological sample is inversely proportional to the amount of labeled rAß(1-42) globulomer standard bound to the anti-Aß(1-42) globulomer antibody.

The antibodies and antibody portions of the invention preferably are capable of neutralizing Aß(1-42) globulomer activity both *in vitro* and *in vivo*. Accordingly, such antibodies and antibody portions of the invention can be used to inhibit Aß(1-42) globulomer activity, *e.g.*, in a cell culture containing Aß(1-42) globulomer, in human subjects, or in other mammalian subjects having Aß(1-42) globulomer with which an antibody of the invention cross-reacts. In one embodiment, the invention provides a method for inhibiting

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6647

Aß(1-42) globulomer activity comprising contacting Aß(1-42) globulomer with an antibody or antibody portion of the invention such that Aß(20-42) globulomer activity is inhibited. For example, in a cell culture containing, or suspected of containing Aß(1-42) globulomer, an antibody or antibody portion of the invention can be added to the culture medium to inhibit Aß(1-42) globulomer activity in the culture.

In another embodiment, the invention provides a method for reducing Aß(1-42) globulomer activity in a subject, advantageously from a subject suffering from a disease or disorder in which Aß(1-42) globulomer activity is detrimental (e.g., an amyloidosis such as Alzheimer's Disease). The invention therefore provides methods for reducing Aß(1-42) globulomer activity in a subject suffering from such a disease or disorder, which method comprises administering to the subject an antibody or antibody portion of the invention such that Aß(1-42) globulomer activity in the subject is reduced. Preferably, the Aß(1-42) globulomer is human Aß(1-42) globulomer, and the subject is a human subject. Alternatively, the subject can be a mammal expressing an Aß(1-42) globulomer to which an antibody of the invention is capable of binding. Still further, the subject can be a mammal into which Aß(1-42) globulomer has been introduced (*e.g.*, by administration of Aß(1-42) globulomer or by expression of Aß(1-42) globulomer transgene). An antibody of the invention can be administered to a human subject for therapeutic purposes. Moreover, an antibody of the invention can be administered to a non-human mammal expressing Aß(1-42) globulomer with which the antibody is capable of binding for veterinary purposes or as an animal model of human disease. Regarding the latter, such animal models may be useful for evaluating the therapeutic efficacy of antibodies of the invention (*e.g.*, testing of dosages and time courses of administration).

As used herein, the term "a disorder in which Aß(1-42) globulomer activity is detrimental" is intended to include

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6648

diseases and other disorders in which the presence of Aß(1-42) globulomer in a subject suffering from the disorder has been shown to be or is suspected of being either responsible for the pathophysiology of the disorder or a factor that contributes to a worsening of the disorder. Accordingly, a disorder in which Aß(1-42) globulomer activity is detrimental is a disorder in which reduction of Aß(1-42) globulomer activity is expected to alleviate some or all of the symptoms and/or progression of the disorder. Such disorders may be evidenced, for example, by an increase in the concentration of Aß(1-42) globulomer in a biological fluid of a subject suffering from the disorder (*e.g.*, an increase in the concentration of Aß(1-42) globulomer in serum, brain tissue, plasma, cerebrospinal fluid, *etc.* of the subject), which can be detected, for example, using an anti-Aß(1-42) globulomer antibody as described above. Non-limiting examples of disorders that can be treated with the antibodies of the invention include those disorders discussed in the section below pertaining to pharmaceutical compositions of the antibodies of the invention.

D. PHARMACEUTICAL COMPOSITION

The invention also provides pharmaceutical compositions comprising an antibody, or antigen-binding portion thereof, of the invention and a pharmaceutically acceptable carrier. The pharmaceutical compositions comprising antibodies of the invention are for use in, but not limited to, diagnosing, detecting, or monitoring a disorder, in preventing, treating, managing, or ameliorating of a disorder or one or more symptoms thereof, and/or in research. In a specific embodiment, a composition comprises one or more antibodies of the invention. In another embodiment, the pharmaceutical composition comprises one or more antibodies of the invention and one or more prophylactic or therapeutic agents other than antibodies of the invention for treating a disorder in which Aß(1-42) globulomer activity is detrimental. Preferably, the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6649

prophylactic or therapeutic agents known to be useful for or having been or currently being used in the prevention, treatment, management, or amelioration of a disorder or one or more symptoms thereof. In accordance with these embodiments, the composition may further comprise of a carrier, diluent or excipient.

The antibodies and antibody-portions of the invention can be incorporated into pharmaceutical compositions suitable for administration to a subject. Typically, the pharmaceutical composition comprises an antibody or antibody portion of the invention and a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible. Examples of pharmaceutically acceptable carriers include one or more of water, saline, phosphate buffered saline, dextrose, glycerol, ethanol and the like, as well as combinations thereof. In many cases, it will be preferable to include isotonic agents, for example, sugars, polyalcohols such as mannitol, sorbitol, or sodium chloride in the composition. Pharmaceutically acceptable carriers may further comprise minor amounts of auxiliary substances such as wetting or emulsifying agents, preservatives or buffers, which enhance the shelf life or effectiveness of the antibody or antibody portion.

Various delivery systems are known and can be used to administer one or more antibodies of the invention or the combination of one or more antibodies of the invention and a prophylactic agent or therapeutic agent useful for preventing, managing, treating, or ameliorating a disorder or one or more symptoms thereof, e.g., encapsulation in liposomes, microparticles, microcapsules, recombinant cells capable of expressing the antibody or antibody fragment, receptor-mediated endocytosis (*see, e.g.*, Wu and Wu, *J. Biol. Chem.* 262:4429-4432 (1987)), construction of a nucleic acid as part of a retroviral or other vector, etc. Methods of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6650

administering a prophylactic or therapeutic agent of the invention include, but are not limited to, parenteral administration (e.g., intradermal, intramuscular, intraperitoneal, intravenous and subcutaneous), epidural administration, intratumoral administration, and mucosal adminsitration (e.g., intranasal and oral routes). In addition, pulmonary administration can be employed, e.g., by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, e.g., U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934, 272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and International Appln. Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In one embodiment, an antibody of the invention, combination therapy, or a composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, MA). In a specific embodiment, prophylactic or therapeutic agents of the invention are administered intramuscularly, intravenously, intratumorally, orally, intranasally, pulmonary, or subcutaneously. The prophylactic or therapeutic agents may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (e.g., oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local.

In a specific embodiment, it may be desirable to administer the prophylactic or therapeutic agents of the invention locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion, by injection, or by means of an implant, said implant being of a porous or non-porous material, including membranes and matrices, such as sialastic membranes, polymers, fibrous matrices (e.g., Tissuel®), or collagen matrices. In one embodiment, an effective amount of one or more antibodies

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6651

of the invention antagonists is administered locally to the affected area to a subject to prevent, treat, manage, and/or ameliorate a disorder or a symptom thereof. In another embodiment, an effective amount of one or more antibodies of the invention is administered locally to the affected area in combination with an effective amount of one or more therapies (e.g., one or more prophylactic or therapeutic agents) other than an antibody of the invention of a subject to prevent, treat, manage, and/or ameliorate a disorder or one or more symptoms thereof.

In another embodiment, the prophylactic or therapeutic agent can be delivered in a controlled release or sustained release system. In one embodiment, a pump may be used to achieve controlled or sustained release (see Langer, supra; Sefton, 1987, *CRC Crit. Ref. Biomed. Eng.* 14:20; Buchwald et al., 1980, *Surgery* 88:507; Saudek et al., 1989, N. Engl. J. Med. 321:574). In another embodiment, polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see e.g., Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, FL (1974); Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York (1984); Ranger and Peppas, 1983, *J. Macromol. Sci. Rev. Macromol. Chem.* 23:61; *see also* Levy et al., 1985, *Science* 228:190; During et al., 1989, *Ann. Neurol.* 25:351; Howard et al., 1989, *J. Neurosurg.* 7 1:105); U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; International Appln. Publication No. WO 99/15154; and International Appln. Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N- vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6652

poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In a preferred embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. In yet another embodiment, a controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (*see, e.g.*, Goodson, in Medical Applications of Controlled Release, supra, vol. 2, pp. 115-138 (1984)).

Controlled release systems are discussed in the review by Langer (1990, *Science* 249:1527-1533). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more therapeutic agents of the invention. *See, e.g.*, U.S. Pat. No. 4,526,938, International Appln. Publication No. WO 91/05548, International Appln. Publication No. WO 96/20698, Ning et al., 1996, "Intratumoral Radioimmunotheraphy of a Human Colon Cancer Xenograft Using a Sustained-Release Gel," *Radiotherapy & Oncology* 39:179-189, Song et al., 1995, "Antibody Mediated Lung Targeting of Long-Circulating Emulsions," *PDA Journal of Pharmaceutical Science & Technology* 50:372-397, Cleek et al., 1997, "Biodegradable Polymeric Carriers for a bFGF Antibody for Cardiovascular Application," Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, and Lam et al., 1997, "Microencapsulation of Recombinant Humanized Monoclonal Antibody for Local Delivery," Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759- 760, each of which is incorporated herein by reference in their entireties.

In a specific embodiment, where the composition of the invention is a nucleic acid encoding a prophylactic or therapeutic agent, the nucleic acid can be administered in vivo to promote expression of its encoded prophylactic or therapeutic agent, by constructing it as part of an appropriate nucleic acid expression vector and administering it so that it becomes intracellular, e.g., by use of a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6653

retroviral vector (see U.S. Pat. No. 4,980,286), or by direct injection, or by use of microparticle bombardment (e.g., a gene gun; Biolistic, Dupont), or coating with lipids or cell-surface receptors or transfecting agents, or by administering it in linkage to a homeobox-like peptide which is known to enter the nucleus (see, e.g., Joliot et al., 1991, *Proc. Natl. Acad. Sci. USA* 88:1864-1868). Alternatively, a nucleic acid can be introduced intracellularly and incorporated within host cell DNA for expression by homologous recombination.

A pharmaceutical composition of the invention is formulated to be compatible with its intended route of administration. Examples of routes of administration include, but are not limited to, parenteral, e.g., intravenous, intradermal, subcutaneous, oral, intranasal (e.g., inhalation), transdermal (e.g., topical), transmucosal, and rectal administration. In a specific embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous, subcutaneous, intramuscular, oral, intranasal, or topical administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lidocaine to ease pain at the site of the injection.

If the compositions of the invention are to be administered topically, the compositions can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, shampoo, spray, aerosol, solution, emulsion, or other form well known to one of skill in the art. See, e.g., Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, PA (1995). For non- sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity preferably greater than water are

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6654

typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams, ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (e.g., preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, preferably in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (e.g., a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well known in the art.

If the method of the invention comprises intranasal administration of a composition, the composition can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (e.g., dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, e.g., gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

If the method of the invention comprises oral administration, compositions can be formulated orally in the form of tablets, capsules, cachets, gelcaps, solutions, suspensions, and the like. Tablets or capsules can be prepared by conventional means with pharmaceutically acceptable excipients such as binding agents (e.g.,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6655

pregelatinised maize starch, polyvinylpyrrolidone, or hydroxypropyl methylcellulose); fillers (e.g., lactose, microcrystalline cellulose, or calcium hydrogen phosphate); lubricants (e.g., magnesium stearate, talc, or silica); disintegrants (e.g., potato starch or sodium starch glycolate); or wetting agents (e.g., sodium lauryl sulphate). The tablets may be coated by methods well-known in the art. Liquid preparations for oral administration may take the form of, but not limited to, solutions, syrups or suspensions, or they may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may be prepared by conventional means with pharmaceutically acceptable additives such as suspending agents (e.g., sorbitol syrup, cellulose derivatives, or hydrogenated edible fats); emulsifying agents (e.g., lecithin or acacia); non-aqueous vehicles (e.g., almond oil, oily esters, ethyl alcohol, or fractionated vegetable oils); and preservatives (e.g., methyl or propyl-p- hydroxybenzoates or sorbic acid). The preparations may also contain buffer salts, flavoring, coloring, and sweetening agents as appropriate. Preparations for oral administration may be suitably formulated for slow release, controlled release, or sustained release of a prophylactic or therapeutic agent(s).

The method of the invention may comprise pulmonary administration, e.g., by use of an inhaler or nebulizer, of a composition formulated with an aerosolizing agent. *See, e.g.*, U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and International Appln. Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entireties. In a specific embodiment, an antibody of the invention, combination therapy, and/or composition of the invention is administered using Alkermes AIR® pulmonary drug delivery technology (Alkermes, Inc., Cambridge, Mass.).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6656

The method of the invention may comprise administration of a composition formulated for parenteral administration by injection (e.g., by bolus injection or continuous infusion). Formulations for injection may be presented in unit dosage form (e.g., in ampoules or in multi-dose containers) with an added preservative. The compositions may take such forms as suspensions, solutions or emulsions in oily or aqueous vehicles, and may contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively, the active ingredient may be in powder form for constitution with a suitable vehicle (e.g., sterile pyrogen-free water) before use. The methods of the invention may additionally comprise of administration of compositions formulated as depot preparations. Such long acting formulations may be administered by implantation (e.g., subcutaneously or intramuscularly) or by intramuscular injection. Thus, for example, the compositions may be formulated with suitable polymeric or hydrophobic materials (e.g., as an emulsion in an acceptable oil) or ion exchange resins, or as sparingly soluble derivatives (e.g., as a sparingly soluble salt).

The methods of the invention encompass administration of compositions formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

Generally, the ingredients of compositions are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the mode of administration is infusion, composition can be dispensed with an infusion bottle containing sterile

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6657

pharmaceutical grade water or saline. Where the mode of administration is by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

In particular, the invention also provides that one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is packaged in a hermetically sealed container such as an ampoule or sachette indicating the quantity of the agent. In one embodiment, one or more of the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention is supplied as a dry sterilized lyophilized powder or water free concentrate in a hermetically sealed container and can be reconstituted (e.g., with water or saline) to the appropriate concentration for administration to a subject. Preferably, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied as a dry sterile lyophilized powder in a hermetically sealed container at a unit dosage of at least 5 mg, more preferably at least 10 mg, at least 15 mg, at least 25 mg, at least 35 mg, at least 45 mg, at least 50 mg, at least 75 mg, or at least 100 mg. The lyophilized prophylactic or therapeutic agents or pharmaceutical compositions of the invention should be stored at between 2° C and 8° C in its original container and the prophylactic or therapeutic agents, or pharmaceutical compositions of the invention should be administered within 1 week, preferably within 5 days, within 72 hours, within 48 hours, within 24 hours, within 12 hours, within 6 hours, within 5 hours, within 3 hours, or within 1 hour after being reconstituted. In an alternative embodiment, one or more of the prophylactic or therapeutic agents or pharmaceutical compositions of the invention is supplied in liquid form in a hermetically sealed container indicating the quantity and concentration of the agent. Preferably, the liquid form of the administered composition is supplied in a hermetically sealed container at least 0.25 mg/ml, more preferably at least

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6658

0.5 mg/ml, at least 1 mg/ml, at least 2.5 mg/ml, at least 5 mg/ml, at least 8 mg/ml, at least 10 mg/ml, at least 15 mg/kg, at least 25 mg/ml, at least 50 mg/ml, at least 75 mg/ml or at least 100 mg/ml. The liquid form should be stored at between 2° C and 8° C in its original container.

The antibodies and antibody portions of the invention can be incorporated into a pharmaceutical composition suitable for parenteral administration. Preferably, the antibody or antibody portions will be prepared as an injectable solution containing 0.1-250 mg/ml antibody. The injectable solution can be composed of either a liquid or lyophilized dosage form in a flint or amber vial, ampule or pre-filled syringe. The buffer can be L-histidine (1-50 mM), optimally 5-10 mM, at pH 5.0 to 7.0 (optimally pH 6.0). Other suitable buffers include but are not limited to, sodium succinate, sodium citrate, sodium phosphate or potassium phosphate. Sodium chloride can be used to modify the toxicity of the solution at a concentration of 0-300 mM (optimally 150 mM for a liquid dosage form). Cryoprotectants can be included for a lyophilized dosage form, principally 0-10% sucrose (optimally 0.5-1.0%). Other suitable cryoprotectants include trehalose and lactose. Bulking agents can be included for a lyophilized dosage form, principally 1-10% mannitol (optimally 2-4%). Stabilizers can be used in both liquid and lyophilized dosage forms, principally 1-50 mM L-Methionine (optimally 5-10 mM). Other suitable bulking agents include glycine, arginine, can be included as 0-0.05% polysorbate-80 (optimally 0.005-0.01%). Additional surfactants include but are not limited to polysorbate 20 and BRIJ surfactants. The pharmaceutical composition comprising the antibodies and antibody-portions of the invention prepared as an injectable solution for parenteral administration, can further comprise an agent useful as an adjuvant, such as those used to increase the absorption, or dispersion of a therapeutic protein (e.g., antibody). A particularly useful adjuvant is hyaluronidase, such as Hylenex® (recombinant human hyaluronidase). Addition

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6659

of hyaluronidase in the injectable solution improves human bioavailability following parenteral administration, particularly subcutaneous administration. It also allows for greater injection site volumes (i.e. greater than 1 ml) with less pain and discomfort, and minimum incidence of injection site reactions. (*See* International Appln. Publication No. WO 04/078140 and U.S. Patent Appln. Publication No. US2006104968, incorporated herein by reference.)

The compositions of this invention may be in a variety of forms. These include, for example, liquid, semi-solid and solid dosage forms, such as liquid solutions (*e.g.*, injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends on the intended mode of administration and therapeutic application. Typical preferred compositions are in the form of injectable or infusible solutions, such as compositions similar to those used for passive immunization of humans with other antibodies. The preferred mode of administration is parenteral (e.g., intravenous, subcutaneous, intraperitoneal, intramuscular). In a preferred embodiment, the antibody is administered by intravenous infusion or injection. In another preferred embodiment, the antibody is administered by intramuscular or subcutaneous injection.

Therapeutic compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high drug concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile, lyophilized powders

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6660

for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and spray-drying that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including, in the composition, an agent that delays absorption, for example, monostearate salts and gelatin.

The antibodies and antibody portions of the present invention can be administered by a variety of methods known in the art, although for many therapeutic applications, the preferred route/mode of administration is subcutaneous injection, intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. In certain embodiments, the active compound may be prepared with a carrier that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are patented or generally known to those skilled in the art. See, *e.g.*, *Sustained and Controlled Release Drug Delivery Systems*, J.R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

In certain embodiments, an antibody or antibody portion of the invention may be orally administered, for example, with an inert diluent or an assimilable edible carrier. The compound (and other ingredients, if desired) may also be enclosed in a hard or soft shell gelatin capsule, compressed into tablets, or incorporated directly into the subject's diet. For oral therapeutic administration, the compounds may

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6661

be incorporated with excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. To administer a compound of the invention by other than parenteral administration, it may be necessary to coat the compound with, or co-administer the compound with, a material to prevent its inactivation.

Supplementary active compounds can also be incorporated into the compositions. In certain embodiments, an antibody or antibody portion of the invention is coformulated with and/or coadministered with one or more additional therapeutic agents that are useful for treating disorders in which Aß(1-42) activity is detrimental. For example, an anti-Aß(1-42) antibody or antibody portion of the invention may be coformulated and/or coadministered with one or more additional antibodies that bind other targets (*e.g.*, antibodies that bind other cytokines or that bind cell surface molecules). Furthermore, one or more antibodies of the invention may be used in combination with two or more of the foregoing therapeutic agents. Such combination therapies may advantageously utilize lower dosages of the administered therapeutic agents, thus avoiding possible toxicities or complications associated with the various monotherapies.

In certain embodiments, an antibody to Aß(1-42)or fragment thereof is linked to a half-life extending vehicle known in the art. Such vehicles include, but are not limited to, the Fc domain, polyethylene glycol, and dextran. Such vehicles are described, e.g., in U.S. Patent Application Serial No. 09/428,082 and published International Patent Application No. WO 99/25044, which are hereby incorporated by reference for any purpose.

In a specific embodiment, nucleic acid sequences comprising nucleotide sequences encoding an antibody of the invention or another prophylactic or therapeutic agent of the invention are administered to treat, prevent, manage, or ameliorate a disorder or one or more symptoms thereof by way

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6662

of gene therapy. Gene therapy refers to therapy performed by the administration to a subject of an expressed or expressible nucleic acid. In this embodiment of the invention, the nucleic acids produce their encoded antibody or prophylactic or therapeutic agent of the invention that mediates a prophylactic or therapeutic effect.

Any of the methods for gene therapy available in the art can be used according to the present invention. For general reviews of the methods of gene therapy, see Goldspiel et al., 1993, *Clinical Pharmacy* 12:488-505; Wu and Wu, 1991, *Biotherapy* 3:87-95; Tolstoshev, 1993, *Ann. Rev. Pharmacol. Toxicol.* 32:573-596; Mulligan, *Science* 260:926- 932 (1993); and Morgan and Anderson, 1993, *Ann. Rev. Biochem.* 62:191-217; May, 1993, *TIBTECH* 11(5):155-215. Methods commonly known in the art of recombinant DNA technology which can be used are described in Ausubel et al. (eds.), Current Protocols in Molecular Biology, John Wiley &Sons, NY (1993); and Kriegler, Gene Transfer and Expression, A Laboratory Manual, Stockton Press, NY (1990). Detailed description of various methods of gene therapy are disclosed in U.S. Patent Application Publication No. US20050042664 A1 which is incorporated herein by reference.

Antibodies of the invention or antigen binding portions thereof can be used alone or in combination to treat diseases such as Alpha1-antitrypsin-deficiency, C1-inhibitor deficiency angioedema, Antithrombin deficiency thromboembolic disease, Kuru, Creutzfeld-Jacob disease/scrapie, Bovine spongiform encephalopathy, Gerstmann-Straussler-Scheinker disease, Fatal familial insomnia, Huntington's disease, Spinocerebellar ataxia, Machado-Joseph atrophy, Dentato-rubro-pallidoluysian atrophy, Frontotemporal dementia, Sickle cell anemia, Unstable hemoglobin inclusion-body hemolysis, Drug-induced inclusion body hemolysis, Parkinson's disease, Systemic AL amyloidosis, Nodular AL amyloidosis, Systemic AA amyloidosis, Prostatic amyloid, Hemodialysis amyloidosis, Hereditary (Icelandic) cerebral angiopathy, Huntington's disease, Familial visceral

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6663

amyloid, Familial visceral polyneuropathy, Familial visceral amyloidosis, Senile systemic amyloidosis, Familial amyloid neurophathy, Familial cardiac amyloid, Alzheimer's disease, Down's syndrome, Medullary carcinoma thyroid and Type 2 diabetes mellitus (T2DM). Preferably, the antibodies of the present invention may be utilized to treat an amyloidosis, for example, Alzheimer's disease and Down's syndrome.

It should be understood that the antibodies of the invention or antigen binding portion thereof can be used alone or in combination with one or more additional agents, e.g., a therapeutic agent (for example, a small molecule or biologic), said additional agent being selected by the skilled artisan for its intended purpose. For example, the additional agent can be a therapeutic agent such as a cholesterinase inhibitor (e.g., tactrine, donepezil, rivastigmine or galantamine), a partial NMDA receptor blocker (e.g., memantine), a glycosaminoglycan mimetic (e.g., Alzhemed), an inhibitor or allosteric modulator of gamma secretase (e.g., R-flurbiprofen), a luteinizing hormone blockade gonadotropin releasing hormone agonist (e.g., leuprorelin), a serotinin 5-HT1A receptor antagonist, a chelatin agent, a neuronal selective L-type calcium channel blocker, an immunomodulator, an amyloid fibrillogenesis inhibitor or amyloid protein deposition inhibitor (e.g., M266) , another antibody (e.g., bapineuzumab), a 5-HT1a receptor antagonist, a PDE4 inhibitor, a histamine agonist, a receptor protein for advanced glycation end products, a PARP stimulator, a serotonin 6 receptor antagonist, a 5-HT4 receptor agonist, a human steroid, a glucose uptake stimulant which enhanceds neuronal metabolism, a selective CB1 antagonist, a partial agonist at benzodiazepine receptors, an amyloid beta production antagonist or inhibitor, an amyloid beta deposition inhibitor, a NNR alpha-7 partial antagonist, a cytokine inhibitor, a TNF antagonist (e.g., Humira and Remicade), a TNF receptor fusion protein (e.g., Enbrel), a therapeutic targeting PDE4, a RNA translation inhibitor, a muscarinic agonist, a nerve growth

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6664

factor receptor agonist, a NGF receptor agonist and a gene therapy modulator (i.e., those agents currently recognized, or in the future being recognized, as useful to treat the disease or condition being treated by the antibody of the present invention). The additional agent also can be an agent that imparts a beneficial attribute to the therapeutic composition e.g., an agent which effects the viscosity of the composition.

It should further be understood that the combinations which are to be included within this invention are those combinations useful for their intended purpose. The agents set forth below are illustrative for purposes and not intended to be limited. The combinations, which are part of this invention, can be the antibodies of the present invention and at least one additional agent selected from the lists below. The combination can also include more than one additional agent, e.g., two or three additional agents if the combination is such that the formed composition can perform its intended function.

The pharmaceutical compositions of the invention may include a "therapeutically effective amount" or a "prophylactically effective amount" of an antibody or antibody portion of the invention. A "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired therapeutic result. A therapeutically effective amount of the antibody or antibody portion may be determined by a person skilled in the art and may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody or antibody portion to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody, or antibody portion, are outweighed by the therapeutically beneficial effects. A "prophylactically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the desired prophylactic result. Typically, since a prophylactic dose is

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6665

used in subjects prior to or at an earlier stage of disease, the prophylactically effective amount will be less than the therapeutically effective amount.

Dosage regimens may be adjusted to provide the optimum desired response (*e.g.*, a therapeutic or prophylactic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. It is especially advantageous to formulate parenteral compositions in dosage unit form for ease of administration and uniformity of dosage. Dosage unit form as used herein refers to physically discrete units suited as unitary dosages for the mammalian subjects to be treated; each unit containing a predetermined quantity of active compound calculated to produce the desired therapeutic effect in association with the required pharmaceutical carrier. The specification for the dosage unit forms of the invention are dictated by and directly dependent on (a) the unique characteristics of the active compound and the particular therapeutic or prophylactic effect to be achieved, and (b) the limitations inherent in the art of compounding such an active compound for the treatment of sensitivity in individuals.

An exemplary, non-limiting range for a therapeutically or prophylactically effective amount of an antibody or antibody portion of the invention is 0.1-20 mg/kg, more preferably 1-10 mg/kg. It is to be noted that dosage values may vary with the type and severity of the condition to be alleviated. It is to be further understood that for any particular subject, specific dosage regimens should be adjusted over time according to the individual need and the professional judgment of the person administering or supervising the administration of the compositions, and that dosage ranges set forth herein are exemplary only and are not intended to limit the scope or practice of the claimed composition.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6666

It will be readily apparent to those skilled in the art that other suitable modifications and adaptations of the methods of the invention described herein are obvious and may be made using suitable equivalents without departing from the scope of the invention or the embodiments disclosed herein. Having now described the present invention in detail, the same will be more clearly understood by reference to the following examples, which are included for purposes of illustration only and are not intended to be limiting of the invention.

EXAMPLE I

GENERATION AND ISOLATION OF HUMANIZED ANTI-Aß(1-42) GLOBULOMER MONOCLONAL ANTIBODIES

This example describes the humanization of an anti-A-beta antibody. Humanization of the murine monoclonal antibody 8F5 (Mu8F5) was carried out essentially according to the procedure of Queen, C., et al., Proc. Natl. Acad. Sci. USA 86: 10029-10033 (1989). First, human V segments with high homology to the Mu8F5 VH or VL amino acid sequences were identified. Next, the complementarity-determining region (CDR) sequences together with framework amino acids important for maintaining the structures of the CDRs were grafted into the selected human framework sequences. In addition, human framework amino acids that were found to be rare in the corresponding V region subgroup were substituted with consensus amino acids to reduce potential immunogenicity. The resulting humanized monoclonal antibody (Hu8F5) was expressed in the human kidney cell line 293T/17. Using a competitive binding assay with purified 8F5 antibodies, the affinity of Hu8F5 to human A-beta was shown to be equivalent to that of Mu8F5.

Materials and Methods

Humanization:

Humanization of the antibody V regions was carried out as outlined by Queen, C., et al., ibid. The human V region

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6667

frameworks used as acceptors for the CDRs of Mu8F5 were chosen based on sequence homology. The computer programs ABMOD and ENCAD (Levitt, M., J. Mol. Biol. 168: 595-620 (1983)) were used to construct a molecular model of the variable regions. Amino acids in the humanized V regions predicted to have contact with the CDRs were substituted with the corresponding residues of Mu8F5. Amino acids in the humanized V region that were found to be rare in the same V region subgroup were changed to consensus amino acids to eliminate potential immunogenicity.

The heavy and light chain variable region genes were designed using approximately 30 overlapping synthetic oligonucleotides ranging in length from approximately 20 to 40 bases following a published method (Rouillard, J.-M., et al., Nucleic Acids Res. 32: W176-W180 (2004)). The oligonucleotides were annealed and assembled with PfuTurbo DNA Polymerase (Stratagene, La Jolla, CA), yielding a full-length product. The resulting product was amplified by the polymerase chain reaction (PCR) using PfuTurbo DNA Polymerase (Stratagene). The PCR-amplified fragments were gel-purified, and cloned into the pCR4Blunt-TOPO vector (Invitrogen Corporation, Carlsbad, CA). After sequence confirmation, Hu8F5 VH and Hu8F5 VL were digested with MluI and XbaI, gel-purified, and subcloned, respectively, into a modified form of pVg1.D.Tt (Cole, M.S., et al., J. Immunol. 159: 3613-3621 (1997); and see below) and pVk (Co, M.S., et al., J. Immunol. 148: 1149-1154 (1992)). The final plasmids were verified by restriction mapping. The sequences of the variable regions of the heavy and light chains were verified by nucleotide sequencing.

Site-directed mutagenesis

Site-directed mutagenesis of the synthetic V-genes was done using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene), following the manufacturer's recommendations. To generate the W47L mutation in the Hu8F5VH gene sequence, a pair of synthetic oligonucleotide primers both containing the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6668

desired mutation was designed. The mutagenesis primers 5'W47L (5' - CTG GCA AGG GCC TGG AGC TGG TGG CCA GCA TCA ACA GCA AC - 3')(SEQ ID NO:31) and 3'W47L (5' - GTT GCT GTT GAT GCT GGC CAC CAG CTC CAG GCC CTT GCC AG - 3') (SEQ ID NO:32) were used. The PCR step was done following the manufacturer's recommendations, by incubating at 95°C for 30 sec, followed by 18 cycles of 95°C for 30 sec, 55°C for 1 min and 68°C for 1 min, followed by incubating at 68°C for 7 min. The oligonucleotide primers, each complementary to opposite strands of the vector were extended by PfuTurbo DNA Polymerase (Stratagene) without primer displacement. The resulting PCR reaction generated a mutated plasmid containing staggered nicks, which was treated with DpnI endonuclease specific for methylated and hemimethylated DNA to digest the parental DNA template and to select for mutation-containing synthesized DNA. The nicked vector DNA incorporating the desired mutations was then transformed into *E. coli* strain TOP10 Chemically Competent Cells (Invitrogen). Sequence verified miniprep DNA was digested with MluI and XbaI, and the resulting restriction fragment containing the mutated Hu8F5VH gene was subcloned into the modified pVg1.D.Tt expression vector described below.

Modification of expression vectors

The allotype of the human gamma-1 constant region gene in the expression plasmid pVg1.D.Tt was modified from G1m (z,a) to the z, non-a allotype. The overlap-extension PCR method (Higuchi, R., in "PCR Technology: Principles and Applications for DNA Amplification", Stockton Press, New York (1989), pp. 61-70) was used to generate the amino acid substitutions D356E and L358M (numbered according to the EU index of Kabat, E.A., et al., "Sequences of Proteins of Immunological Interest", 5th ed., National Institutes of Health, Bethesda, MD (1991)), using the mutagenesis primers 356E358M-A (5' - CCA TCC CGG GAG GAG ATG ACC AAG AAC - 3')(SEQ ID NO:33) and 356E358M-B (5' - GTT CTT GGT CAT CTC CTC CCG GGA TGG - 3')(SEQ ID NO:34). The

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6669

first round of PCR used outside primer g1-5 (5' - CCA CAT GGA CAG AGG CCG - 3')(SEQ ID NO:35) and 356E358M-B for the left-hand fragment, and outside primer mc-124 (5' - AGG GCA GCG CTG GGT GC - 3')(SEQ ID NO:36) and 356E358M-A for the right-hand fragment. The PCR reactions were done using the Expand High Fidelity PCR System (Roche Diagnostics Corporation, Indianapolis, IN) by incubating at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 1 min, followed by incubating at 72°C for 10 min. The second round of PCR to combine the left-hand and right-hand fragments was done as described above, using outside primers g1-5 and mc-124, by incubating at 95°C for 5 min, followed by 35 cycles of 95°C for 30 sec, 60°C for 30 sec and 72°C for 90 sec, followed by incubating at 72°C for 7 min. Following digestion with SfiI and EagI, the resulting restriction fragment was subcloned into a modified form of the pVg1.D.Tt expression vector containing an NheI restriction site in the intron between the hinge and CH2 exons.

Mutations to the lower hinge region of the gamma-1 constant region gene were also generated by site-directed mutagenesis, using the plasmid described above as a template. To generate the amino acid substitutions L234A and L235A (numbered according to the EU index of Kabat, E.A., et al., ibid.), the mutagenesis primers 5'L234A L235A (5' - CAT CTC TTC CTC AGC ACC TGA AGC CGC GGG GGG ACC GTC AGT CTT CCT - 3')(SEQ ID NO:37) and 3'L234A L235A (5' - AGG AAG ACT GAC GGT CCC CCC GCG GCT TCA GGT GCT GAG GAA GAG ATG - 3')(SEQ ID NO:38) were used. The PCR step was done using the QuikChange II Site-Directed Mutagenesis Kit (Stratagene), as described above, by incubating at 95°C for 30 sec, followed by 18 cycles of 95°C for 30 sec, 55°C for 1 min and 68°C for 1 min, followed by incubating at 68°C for 7 min. Following digestion with DpnI, *E. coli* strain TOP10 Chemically Competent Cells (Invitrogen) were transformed with a small portion of the PCR product. The plasmid was digested with NheI and EagI, and the resulting restriction fragment was subcloned into the modified

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6670

pVg1.D.Tt expression vector described above containing an NheI site in the intron between the hinge and CH2 exons. Mutations were verified by nucleotide sequencing.

Transient transfection

Human kidney cell line 293T/17 (American Type Culture Collection, Manassus, VA) was maintained in DMEM (BioWhittaker, Walkersville, MD) containing 10% Fetal Bovine Serum (FBS) (HyClone, Logan, UT), 0.1 mM MEM non-essential amino acids (Invitrogen) and 2 mM L-glutamine (Invitrogen), hereinafter referred to as 293 medium, at 37°C in a 7.5% CO_2 incubator. For expression and purification of monoclonal antibodies after transient transfection, 293T/17 cells were incubated in DMEM containing 2% low-IgG FBS (HyClone), 0.1 mM MEM non-essential amino acids and 2 mM L-glutamine, hereinafter referred to as low-IgG 293 medium.

Transient transfection of 293T/17 cells was carried out using Lipofectamine 2000 (Invitrogen) following the manufacturer's recommendations. Approximately 2 x 10^7 cells per transfection were plated in a T-175 flask in 50 ml of 293 medium and grown overnight to confluence. The next day, 35 µg of light chain plasmid and 35 µg of heavy chain plasmid were combined with 3.75 ml of Hybridoma-SFM (HSFM) (Life Technologies, Rockville, MD). In a separate tube, 175 µl of Lipofectamine 2000 reagent and 3.75 ml of HSFM were combined and incubated for 5 min at room temperature. The 3.75 ml Lipofectamine 2000-HSFM mixture was mixed gently with the 3.75 ml DNA-HSFM mixture and incubated at room temperature for 20 min. The medium covering the 293T/17 cells was aspirated and replaced with low-IgG 293 medium, then the lipofectamine-DNA complexes were added dropwise to the cells, mixed gently by swirling, and the cells were incubated for 7 days at 37°C in a 7.5% CO_2 incubator before harvesting the supernatants.

Measurement of antibody expression by ELISA

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6671

Expression of Hu8F5 antibodies was measured by sandwich ELISA. MaxiSorp ELISA plates (Nunc Nalge International, Rochester, NY) were coated overnight at 4°C with 100 µl/well of a 1:1000 dilution of AffiniPure goat anti-human IgG Fcγ-chain specific polyclonal antibodies (Jackson ImmunoResearch Laboratories, Inc., West Grove, PA) in 0.2 M sodium carbonate-bicarbonate buffer, pH 9.4, washed with Wash Buffer (PBS containing 0.1% Tween 20), and blocked for 1 hr at room temperature with 300 µl/well of SuperBlock Blocking Buffer in TBS (Pierce Chemical Company, Rockford, IL). After washing with Wash Buffer, samples containing Hu8F5 were appropriately diluted in ELISA Buffer (PBS containing 1% BSA and 0.1% Tween 20) and 100 µl/well was applied to the ELISA plates. As a standard, humanized IgG1/κ antibody daclizumab (PDL BioPharma, Inc.) was used. After incubating the plates for 1 hr at room temperature, and washing with Wash Buffer, bound antibodies were detected using 100 µl/well of a 1:1000 dilution of HRP-conjugated goat anti-human kappa light chain specific polyclonal antibodies (Southern Biotechnology Associates, Inc., Birmingham, AL). After incubating for 1 hr at room temperature, and washing with Wash Buffer, color development was performed by adding 100 µl/well of ABTS Peroxidase Substrate/Peroxidase Solution B (KPL, Inc., Gaithersburg, MD). After incubating for 7 min at room temperature, color development was stopped by adding 50 µl/well of 2% oxalic acid. Absorbance was read at 415 nm using a VersaMax microplate reader (Molecular Devices Corporation, Sunnyvale, CA).

Purification of 8F5 antibodies

Culture supernatants from transient transfections were harvested by centrifugation, and sterile filtered. The pH of the filtered supernatants was adjusted by addition of 1/50 volume of 1 M sodium citrate, pH 7.0. Supernatants were run over a 1 ml HiTrap Protein A HP column (GE Healthcare Bio-Sciences Corporation, Piscataway, NJ) that was pre-

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6672

equilibrated with 20 mM sodium citrate, 150 mM NaCl, pH 7.0. The column was washed with the same buffer, and bound antibody was eluted with 20 mM sodium citrate, pH 3.0. After neutralization by addition of 1/50 volume of 1.5 M sodium citrate, pH 6.5, the pooled antibody fractions were concentrated to ~0.5-1.0 mg/ml using a 15 ml Amicon Ultra-15 centrifugal filter device (30,000 dalton MWCO) (Millipore Corporation, Bedford, MA). Samples were then filter sterilized using a 0.2 µm Acrodisc syringe filter with HT Tuffryn membrane (Pall Corporation, East Hills, NY). The concentrations of the purified antibodies were determined by UV spectroscopy by measuring the absorbance at 280 nm (1 mg/ml = 1.4 A_{280}).

Competition ELISA

MaxiSorp ELISA plates (Nalge Nunc International) were coated overnight at 4°C with 100 µl/well of 5.0 µg/ml human A-beta oligomer antigen (1-42) (Abbott Biosciences Corporation, Worcester, MA) in PBS, washed with Wash Buffer (PBS containing 0.1% Tween 20), and blocked for 1 hr at room temperature with 340 µl/well of SuperBlock Blocking Buffer in PBS (Pierce Chemical Company). After washing with Wash Buffer, a mixture of biotinylated Mu8F5 (1.0 µg/ml final concentration) and competitor antibody (Mu8F5 or Hu8F5 starting at 27 µg/ml final concentration and serially diluted 3-fold) in 100 µl/well of 5% Superblock Blocking Buffer in PBS was added in triplicate. As a no-competitor control, 100 µl/well of 5% Superblock Blocking Buffer in PBS was used. After incubating the plates for 2 hrs at room temperature, and washing with Wash Buffer, bound antibodies were detected using 100 µl/well of 1 µg/ml HRP-conjugated streptavidin (Pierce Chemical Company) in 5% Superblock Blocking Buffer in PBS. After incubating for 30 min at room temperature, and washing with Wash Buffer, color development was performed by adding 100 µl/well of ABTS Peroxidase Substrate/Peroxidase Solution B (KPL). After incubating for 5 min at room temperature, color development

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6673

was stopped by adding 50 µl/well of 2% oxalic acid. Absorbance was read at 415 nm.

Results:

Humanization

For humanization of the Mu8F5 variable regions, the general approach provided in the present invention was followed. First, a molecular model of the Mu8F5 variable regions was constructed with the aid of the computer programs ABMOD and ENCAD (Levitt, M., J. Mol. Biol. 168: 595-620 (1983)). Next, based on a homology search against human V and J segment sequences, the VH segment YSE'CL (Mariette, X., et al., Eur. J. Immunol. 23: 846-851 (1993)) and the J segment JH4 (Ravetch, J.V., et al., Cell 27: 583-591 (1981)) were selected to provide the frameworks for the Hu8F5 heavy chain variable region. For the Hu8F5 light chain variable region, the VL segment TR1.37'CL (Portolano, S., et al., J. Immunol. 151: 2839-2851 (1993)) and the J segment JK4 (Hieter, P.A., et al., J. Biol. Chem. 257: 1516-1522 (1982)) were used. The identity of the framework amino acids between Mu8F5 VH and the acceptor human YSE'CL and JH4 segments was 80%, while the identity between Mu8F5 VL and the acceptor human TR1.37'CL and JK4 segments was 86%.

At framework positions in which the computer model suggested significant contact with the CDRs, the amino acids from the mouse V regions were substituted for the original human framework amino acids. This was done at residues 49 and 98 for versions 1 and 2 of the heavy chain, and additionally at residue 47 for version 2 of the heavy chain (Fig. 7). For the light chain, replacement was made at residue 50 (Fig. 8). Framework residues that occurred only rarely at their respective positions in the corresponding human V region subgroups were replaced with human consensus amino acids at those positions. This was done at residues 13 and 78 of the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6674

heavy chain (Fig. 4), and at residues 1 and 2 of the light chain (Fig. 5).

Expression of the Hu8F5 antibodies

Genes encoding humanized VH or VL were designed as mini-exons including signal peptides, splice donor signals, and appropriate restriction enzyme sites for subsequent cloning into a mammalian expression vector. The splice donor signals in the VH and VL mini-exons were derived from the corresponding human germline JH and JK sequences, respectively. The signal peptide sequences in the humanized VH and VL mini-exons were provided by Abbott Bioresearch Center. The Hu8F5 VH and VL genes were constructed by assembly of overlapping synthetic oligonucleotides and PCR. Primers 1-28 for the synthesis of the humanized heavy chain variable region are presented in Table A. Primers 1-29 for the synthesis of the humanized light chain variable region are presented in Table B.

The DNA sequences and deduced amino acid sequences of the humanized VHv1, VHv2 and VL mini-exons are shown in Figs. 6, 7 and 8, respectively. The resulting V gene fragments were cloned, respectively, into a modified form of pVg1.D.Tt and pVk (Fig. 9).

Transient transfectants producing Hu8F5 were generated as described in *Materials and Methods*. Culture supernatants of transiently transfected 293T/17 cells were analyzed by ELISA for production of Hu8F5. Expression levels of approximately 30-50 µg/ml were typically observed. Hu8F5 IgG1/κ monoclonal antibodies were purified from exhausted culture supernatant with a protein A Sepharose column as described in *Materials and Methods*. SDS-PAGE analysis under non-reducing conditions indicated that the Hu8F5 antibodies had a molecular weight of about 150-160 kDa. Analysis under reducing conditions indicated that the Hu8F5 antibodies were comprised of a heavy chain with a molecular weight of about 50 kDa and a light

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6675

chain with a molecular weight of about 25 kDa. The purity of the antibodies appeared to be more than 95%.

Binding properties of Hu8F5 antibodies

The affinity of Hu8F5 to human A-beta oligomer antigen (1-42) was analyzed by competition ELISA as described in *Materials and Methods*. A representative result is shown in Fig. 10. Both Mu8F5 and Hu8F5 competed with biotinylated Mu8F5 in a concentration-dependent manner. As shown in Table C, the mean IC_{50} values of Mu8F5, Hu8F5v1 and Hu8F5v2, obtained using the computer software GraphPad Prism (GraphPad Software Inc., San Diego, CA), were 5.08, 6.36 and 6.99 µg/ml, respectively. The binding of Hu8F5v1 and Hu8F5v2 to human A-beta oligomer antigen (1-42) was equivalent to that of Mu8F5. These results clearly indicate that humanization of mouse anti-A-beta monoclonal antibody 8F5 was successful: Hu8F5 retained full binding affinity to human A-beta oligomer antigen (1-42).

TABLE A

Oligo #	**Sequences**
Hu8F5VHv1 #1	agacgctgttgcctTATACGCGTCCACCATGGAGTTCG (SEQ ID NO:39)
Hu8F5VHv1 #2	ACAGCCAGCTCAGGCCGAACTCCATGGTGGACG (SEQ ID NO:40)
Hu8F5VHv1 #3	GCCTGAGCTGGCTGTTCCTGGTGGCCATCC (SEQ ID NO:41)
Hu8F5VHv1 #4	ACTGCACGCCCTTCAGGATGGCCACCAGGA (SEQ ID NO:42)
Hu8F5VHv1 #5	TGAAGGGCGTGCAGTGCGAGGTGCAGCTGG (SEQ ID NO:43)
Hu8F5VHv1 #6	GCCGCCGCTCTCCACCAGCTGCACCTCGC (SEQ ID NO:44)
Hu8F5VHv1 #7	TGGAGAGCGGCGGCGGCCTGGTGCAGCC (SEQ ID NO:45)
Hu8F5VHv1 #8	CAGGCTGCCGCCAGGCTGCACCAGGCC (SEQ ID NO:46)
Hu8F5VHv1 #9	TGGCGGCAGCCTGCGCCTGAGCTGCGC (SEQ ID NO:47)
Hu8F5VHv1 #10	TGAAGCCGCTGGCGGCGCAGCTCAGGCG (SEQ ID NO:48)
Hu8F5VHv1 #11	CGCCAGCGGCTTCACCTTCAGCAGCTACGGC

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6676

	(SEQ ID NO:49)
Hu8F5VHv1 #12	GCGCACCCAGCTCATGCCGTAGCTGCTGAAGG (SEQ ID NO:50)
Hu8F5VHv1 #13	ATGAGCTGGGTGCGCCAGGCCCCTGGCA (SEQ ID NO:51)
Hu8F5VHv1 #14	CCCACTCCAGGCCCTTGCCAGGGGCCTG (SEQ ID NO:52)
Hu8F5VHv1 #15	AGGGCCTGGAGTGGGTGGCCAGCATCAACAGC (SEQ ID NO:53)
Hu8F5VHv1 #16	TGCTGCCGCCGTTGCTGTTGATGCTGGCCA (SEQ ID NO:54)
Hu8F5VHv1 #17	AACGGCGGCAGCACCTACTACCCTGACAGCG (SEQ ID NO:55)
Hu8F5VHv1 #18	TGAAGCGGCCCTTCACGCTGTCAGGGTAGTAGG (SEQ ID NO:56)
Hu8F5VHv1 #19	TGAAGGGCCGCTTCACCATCAGCCGCGACA (SEQ ID NO:57)
Hu8F5VHv1 #20	CAGGGTGTTCTTGGCGTTGTCGCGGCTGATGG (SEQ ID NO:58)
Hu8F5VHv1 #21	ACGCCAAGAACACCCTGTACCTGCAGATGAACAGCCT (SEQ ID NO:59)
Hu8F5VHv1 #22	TGTCCTCGGCGCGCAGGCTGTTCATCTGCAGGTA (SEQ ID NO:60)
Hu8F5VHv1 #23	GCGCGCCGAGGACACCGCCGTGTACTACTGCG (SEQ ID NO:61)
Hu8F5VHv1 #24	AGTAGTCGCCGCTGGCGCAGTAGTACACGGCGG (SEQ ID NO:62)
Hu8F5VHv1 #25	CCAGCGGCGACTACTGGGGCCAGGGCACC (SEQ ID NO:63)
Hu8F5VHv1 #26	TGAGGAGACGGTGACGAGGGTGCCCTGGCCCC (SEQ ID NO:64)
Hu8F5VHv1 #27	CTCGTCACCGTCTCCTCAGGTGAGTCCTCACAACCTC (SEQ ID NO:65)
Hu8F5VHv1 #28	gcgtcacggggtaaATATCTAGAGGTTGTGAGGACTCACC (SEQ ID NO:66)
5' Hu8F5VHv1	agacgctgttgcctTATACG (SEQ ID NO:67)
3' Hu8F5VHv1	gcgtcacggggtaaATATCTA (SEQ ID NO:68)

TABLE B

Oligo #	**Sequences**
Hu8F5VL #1	GCGTATAtcccggttgttgct (SEQ ID NO:69)
Hu8F5VL #2	agcaacaaccgggaTATACGCGTCCACCATGGACATGCG (SEQ ID NO:70)
Hu8F5VL #3	GCTGGGCAGGCACGCGCATGTCCATGGTGGAC (SEQ ID NO:71)
Hu8F5VL #4	CGTGCCTGCCCAGCTGCTGGGCCTGCTG

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6677

	(SEQ ID NO:72)
Hu8F5VL #5	CCAGGGAACCACAGCAGCAGCAGGCCCAGCA (SEQ ID NO:73)
Hu8F5VL #6	CTGCTGTGGTTCCCTGGCAGCCGCTGCGACA (SEQ ID NO:74)
Hu8F5VL #7	GCTCTGGGTCATCACGATGTCGCAGCGGCTG (SEQ ID NO:75)
Hu8F5VL #8	TCGTGATGACCCAGAGCCCTCTGAGCCTGCCTG (SEQ ID NO:76)
Hu8F5VL #9	GCTCGCCAGGGGTCACAGGCAGGCTCAGAGG (SEQ ID NO:77)
Hu8F5VL #10	TGACCCCTGGCGAGCCTGCCAGCATCAGCTGC (SEQ ID NO:78)
Hu8F5VL #11	GCTCTGGCTGCTGCGGCAGCTGATGCTGGCAG (SEQ ID NO:79)
Hu8F5VL #12	CGCAGCAGCCAGAGCCTGGTGTACAGCAACGGC (SEQ ID NO:80)
Hu8F5VL #13	CCAGTGCAGGTAGGTGTCGCCGTTGCTGTACACCAG (SEQ ID NO:81)
Hu8F5VL #14	GACACCTACCTGCACTGGTACCTGCAGAAGCCTGG (SEQ ID NO:82)
Hu8F5VL #15	GCAGCTTAGGGCTCTGGCCAGGCTTCTGCAGGTA (SEQ ID NO:83)
Hu8F5VL #16	CCAGAGCCCTAAGCTGCTGATCTACAAAGTGAGCAACCG (SEQ ID NO:84)
Hu8F5VL #17	GGCACGCCGCTGAAGCGGTTGCTCACTTTGTAGATCA (SEQ ID NO:85)
Hu8F5VL #18	CTTCAGCGGCGTGCCTGACCGCTTCAGCGG (SEQ ID NO:86)
Hu8F5VL #19	TGCCGCTGCCGCTGCCGCTGAAGCGGTCA (SEQ ID NO:87)
Hu8F5VL #20	CAGCGGCAGCGGCACCGACTTCACCCTGAAGA (SEQ ID NO:88)
Hu8F5VL #21	CTCCACGCGGCTGATCTTCAGGGTGAAGTCGG (SEQ ID NO:89)
Hu8F5VL #22	TCAGCCGCGTGGAGGCCGAGGACGTGGG (SEQ ID NO:90)
Hu8F5VL #23	TGGCTGCAGTAGTACACGCCCACGTCCTCGGC (SEQ ID NO:91)
Hu8F5VL #24	CGTGTACTACTGCAGCCAGAGCACCCACGTGCC (SEQ ID NO:92)
Hu8F5VL #25	CCGCCGAAGGTCCAAGGCACGTGGGTGCTC (SEQ ID NO:93)
Hu8F5VL #26	TTGGACCTTCGGCGGCGGCACCAAAGTGGAGA (SEQ ID NO:94)
Hu8F5VL #27	AGGAAAGTGCACTTACGTTTGATCTCCACTTTGGTGCCG (SEQ ID NO:95)
Hu8F5VL #28	TCAAACGTAAGTGCACTTTCCTAATCTAGATATtcggctcgacg (SEQ ID NO:96)
Hu8F5VL #29	cgtcgagccgaATATCTAGATT (SEQ ID NO:97)
5' Hu8F5VL	agcaacaaccgggaTATACGC

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6678

	(SEQ ID NO:98)
3' Hu8F5VL	cgtcgagccgaATATCTAGATT (SEQ ID NO:99)

TABLE C

Antibody	Expt. 1	Expt. 2	Expt. 3	Average	S.D.
Mu8F5	4.58	4.87	5.81	5.08	0.64
Hu8F5v1	6.28	6.85	5.93	6.36	0.47
Hu8F5v2	6.29	8.12	6.56	6.99	0.99

Assembly of humanized antibody VH and VL fragments

VH and VL gene fragments for humanization designs were assembled by annealing overlapping oligonucleotides covering the entire sequence. Briefly, the entire coding strand of the VH or VL fragment was divided into a series of sixty-nucleotide oligos., each designed to have a thirty nucleotide overlap with two corresponding bottom strand oligos. The sum of the bottom strand oligos. also covered the entire sequence. Taken together, the oligonucleotides filled the complete double-stranded DNA segment.

In the first step of the procedure, the oligonucleotides were kinased (New England Biolabs cat #201S) by combining seven top strand and seven bottom strand oligos together at a concentration of 3 nM each in a 100 microliter reaction for 30 minutes at 37 ^{0}C. The kinased oligos were then phenol/chloroform extracted, precipitated, and resuspended in 100 microliters of NEB Ligase Buffer.

In the second step of the procedure, the oligonucleotides were annealed by heating to 95^{0}C, then slowly cooled to 20^{0}C over a period of 90 minutes by a controlled cooling ramp in a PCR machine.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6679

In the third step of the procedure, 1 microliter of Ligase (NEB cat#202S) was added to the annealed oligos in order to ligate them together to form the strands of the VH and VL segments. Ligase was inactivated by heating to 65 ^{0}C for 10 minutes.

In the fourth step, the ends of the assembled fragments were filled in with Klenow enzyme (NEB cat#212S), and the DNA was gel purified before cloning into the human heavy and light chain cassette vectors.

EXAMPLE II

COMPETITION ELISA

The following protocol was utilized to carry out the Competition ELISA assay:

Initially, plates (1 plate/experiment) were coated overnight with A-Beta antigen (1-42) at a concentration of 5 µg/mL in phosphate buffered saline (PBS). The following day, the supernatant was discarded, and the plates were blocked with 340 mL of Super Block buffer (Pierce, Rockford, IL) for 45 min. The plates were then emptied, and the biotinylated 7C6 or 5F7 mouse antibody was added at a concentration of 1 µg/mL. (Volume = 100 µL) Other antibodies (mouse or humanized) were added at concentrations ranging from 27 µg/mL to 0.11 µg/mL. (Volume = 50 µL) The plates were then incubated for two hours and washed 5X times with Phosphate Buffered Saline (PBS). Neutra Avidin HRP was added as a secondary reagent (dilution 1:20,000; volume = 100 µL). The plates were then incubated for 30 min. and washed 5X times. TMB substrate (Invitrogen, Carlsbad, CA) was then added (volume = 100 µL). Subsequently, the plates were incubated for 4 min. The reaction was then stopped with 2N sulfuric acid. (Vol-100 µL) Plates were read spectrophotometrically at a wavelength of 450 nm. The results are shown in Figure 3.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6680

In particular, Figure 3 illustrates the equivalence of the humanized antibody (i.e., 8F5hum8, HUM8) to the mouse parent with respect to the humanized antibody's ability to compete with (and inhibit the binding signal of) the biotinylated mouse antibody. Thus, the humanized antibody has retained its binding potency.

EXAMPLE III

BINDING OF 8F5hum8 TO Aß(1-42) FIBRILS

Since 8F5hum8 antibody was generated against soluble globulomers, it was hypothesized that 8F5hum8 should not bind to deposited plaque or fibril material. Therefore, binding of 8F5hum8 to polymerized Aß fibril suspensions was tested as described in the following example:

- Preparation of Aß(1-42) fibrils:

1mg Aß(1-42) (Bachem Inc., Catalog Nr.: H-1368) was dissolved in 500µl aqueous 0.1% NH_4OH (Eppendorf tube), and the sample was stirred for 1 min at room temperature followed by 5 min centrifugation at 10000 g. Supernatant was pipetted into a new Eppendorf tube and the Aß(1-42) concentration measured according to Bradford protein concentration assay (BIO-RAD Inc. assay procedure).

100µl of this freshly prepared Aß(1-42) solution were neutralized with 300µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 followed by 2% HCl to adjust pH 7.4. The sample was incubated for another 20 hrs at 37°C and centrifuged (10min, 10000g). The supernatant was discarded and the fibril pellet resuspended with 400µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 under 1 min stirring on a Vortex mixer followed by centrifugation (10min, 10000g). After discarding the supernatant, this resuspending procedure was repeated, and the final fibril suspension spun down by another centrifugation (10min, 10000g). The supernatant was once again discarded and the final pellet resuspended in 380µl 20mM NaH2PO4; 140mM NaCl;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6681

pH7.4 under 1 min stirring on a Vortex mixer. Aliquots of the sample were stored at -20°C in a freezer.

80µl fibril suspension were mixed with 320µl 20mM NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4, buffer and stirred for 5 min at room temperature followed by sonification (20 sec). After centrifugation (10min, 10000g), the pellet was resuspended with 190µl 20mM NaH2PO4; 140mM NaCl; 0.05% Tween 20; pH 7.4 under stirring in a Vortex mixer.

- Binding of Antibodies to Aß(1-42) fibrils

10µl aliquots of this fibril suspension was incubated with:

a) 10µl 20mM Na Pi; 140mM NaCl; pH 7.4
b) 10µl 0.1µg/µl mMAb 6E10 Signet Inc. Cat.#9320 in 20mM NaH2PO4; 140mM NaCl; pH 7.4
c) 10µl 0.1µg/µl mMAb 8F5hum8 in 20mM Na Pi; 140mM NaCl; pH 7.4
d) 10µl 0.1µg/µl mMAb IgG2a in 20mM Na Pi; 140mM NaCl; pH 7.4

Samples were incubated for 20h at 37°C. Finally the samples were centrifuged (10 min at 10000g). The supernatants containing the unbound antibody fraction were collected and mixed with 20 µl SDS-PAGE sample buffer. The pellet fractions were washed with 50µl 20mM NaH2PO4; 140mM NaCl; pH 7.4 buffer under 1 min stirring in a Vortex mixer followed by centrifugation (10min, 10000g). The final pellets were resuspended in 20µl 20mM Na Pi; 140mM NaCl; 0.025% Tween 20; pH 7.4 buffer and solved in 20µl SDS-PAGE buffer.

-SDS-PAGE analysis

Supernatants and resuspended pellet samples were heated for 5 min at 98°C and loaded onto a 18% Tris/Glycin Gel under the following conditions:

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6682

SDS-sample buffer: 0.3g SDS; 0.77g DTT; 4ml 1M Tris/HCl pH 6.8; 8ml glycerol; 1ml 1% Bromphenolblue in Ethanol; add water to 50 ml 18% Tris/Glycin Gel:Invitrogen Inc., No.: EC6505BOX running buffer: 7.5g Tris; 36g Glycine; 2.5g SDS; add water to 2.5 l

The PAGE was run at 20 mA. Gels were stained by Coomassie Blue R250.

Results:

Coomassie staining of SDS-PAGE indicated the presence of heavy and light chains of antibodies predominantly in the supernatant of the fibril suspension for the antibody 8F5hum8 (lane 3, Figure 2a), the remaining fibril suspension of the pellet showed very little antibody material while also showing partly depolymerized Abeta at 4.5 kDa. In contrast to 8F5hum8, other anti-Aß antibodies did not show up in the soluble fraction (6E10, lane 5, Figure 2a) compared to fibril bound fraction (lane 6, Figure 2a). As a reference for unspecific binding and the intrinsic background of this method, the unspecific antibody IgG2a was used as an internal control. The IgG2a antibody, which is not directed against the Aß peptide in any form, shows a certain unspecific binding to Aß fibrils.

The relative binding to fibril type Abeta was evaluated from SDS-PAGE analysis by measuring the Reflective Density values from the heavy chain of the antibodies in the fibril bound and the supernatant fractions and calculated according to the following formula:

Fibril bound Ab fraction = $RD_{\text{fibril faction}}$ x100%/($RD_{\text{fibril faction}}$ + $RD_{\text{supernatant fraction}}$).

The following values were obtained:

Antibody	Fibril bound Ab fraction

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6683

6E10	91%
8F5hum8	20%
IgG2a	9%

These data indicate a significant reduction of bound 8F5hum8 compared to standard antibody 6E10.

EXAMPLE IV

8F5HUM8 PREFERENTIAL GLOBULOMER BINDING COMPARED TO MONOMER PREPARATIONS OF AB(1-40) AND AB(1-42) DETERMINED BY DOT BLOT

To test the selectivity of 8F5hum8, Aß(1-42) monomer as well as freshly prepared Aß(1-40) were used as surrogates for monomers. The oligomer selectivity versus Aß(1-42) monomer and Aß(1-40) monomer was examined by dot blot immunoassay. In this experiment, 8F5hum8 exhibited preferential binding to Aß(1-42) globulomer (compared to a known antibody 4G8 mapping to a similar region as 8F5hum8, but derived from immunization with a linear peptide Aβ(17-24) (Abcam Ltd., Cambridge, MA)), as compared to Aß(1-42) monomer as well as compared to Aß(1-40) monomer.

Description of Dot blot method

1) Preparation of Aß antigens:

a) Preparation of Aß(1-42) globulomer:

9 mg Aß(1-42) Fa. Bachem were dissolved in 1.5ml HFIP (1.1.1.3.3.3 Hexafluor-2-propanol) and incubated 1,5 h at 37°C. The solution was evaporated in a SpeedVac and suspended in 396 µl DMSO (5mM Aß stock solution). The sample was sonified for 20 seconds in a sonic water bath, shaken for 10 minutes and stored over night at -20°C.

The sample was diluted with 4.5 ml PBS (20 mM NaH2PO4; 140 mM NaCl; pH 7,4) and 0.5 ml 2 % aqueous SDS-solution were added

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6684

(0.2% SDS content). The mixture was incubated for 7 h at 37°C, diluted with 16 ml H_2O and further incubated for 16 hours at 37 deg C. After that, the Aß(1-42) globulomer solution was centrifuged for 20min at 3000g. The supernatant was concentrated to 0.5ml by 30KDa centriprep. The concentrate was dialysed against 5mM NaH2PO4; 35mM NaCl; pH7.4 overnight at 6°C. Subsequently, the Aß(1-42) globulomer concentrate was centrifuged for 10min at 10000g. The supernatant was then aliquoted and stored at -20°C.

b) Preparation of monomer Aß(1-42), HFIP pretreated:

3mg human Aß(1-42),(Bachem Inc) cat. no. H-1368 were dissolved in 0.5ml HFIP (6mg/ml suspension) in an 1.7 ml Eppendorff tube and was shaken (Eppendorff Thermo mixer, 1400 rpm) for 1.5h at 37°C till a clear solution was obtained. The sample was dried in a speed vac concentrator (1.5h) and resuspended in 13.2µl DMSO, shook for 10 sec., followed by ultrasound bath sonification (20 sec) and shaking (e.g. in Eppendorff Thermo mixer, 1400 rpm) for 10 min.

6ml 20mM NaH2PO4; 140mM NaCl; 0.1% Pluronic F68; pH 7.4 was added and stirred for 1h at room temperature. The sample was centrifuged for 20min at 3000g. The supernatant was discarded and the precipitate solved in 0.6ml 20mM NaH2PO4; 140mM NaCl; 1% Pluronic F68; pH 7.4. 3.4ml water was added and stirred for 1h at room temperature followed by 20 min centrifugation at 3000 g. 8 x 0.5ml aliquots of the supernatant were stored at -20°.

c) Preparation of monomer Aß(1-40):

1mg human Aß(1-40), (Bachem Inc) cat. no. H-1194 was suspended in 0.25ml HFIP (4mg/ml suspension) in an Eppendorff tube. The tube was shaken (e.g., in an Eppendorff Thermo

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6685

mixer, 1400 rpm) for 1.5h at 37°C to get a clear solution and afterwards dried in a speed vac concentrator(1.5h). The sample was redissolved in 46µl DMSO (21.7 mg/ml solution), shaken for 10 sec., followed by 20 sec. sonification in ultrasound bath. After 10 min of shaking (e.g. in Eppendorff Thermo mixer, 1400 rpm), the sample was stored at -20°C for further use.

2) Dot blot: Materials and procedure:

Materials for dot blot:

Aβ-standards:

Serial dilution of Aβ antigens in 20 mM NaH2PO4, 140 mM NaCl, pH 7.4 + 0.2 mg/ml BSA

1) 100 pmol/µl
2) 10 pmol/µl
3) 1 pmol/µl
4) 0,1 pmol/µl
5) 0,01 pmol/µl

Nitrocellulose:

Trans-Blot Transfer medium, Pure Nitrocellulose Membrane (0.45 µm); BIO-RAD

Anti-Mouse-AP:

Cat no: AP326A (Chemicon)

Anti-human-AP:

Cat no: A3313 (Sigma)

Detection reagent:

NBT/BCIP; Cat no: 11697471001; Roche)

Bovine Serum Albumin, (BSA):

Cat no: A-7888 (SIGMA)

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6686

Blocking reagent:

5 % low fat milk in TBS

Buffer solutions:

TBS
25 mM Tris / HCl buffer pH 7.5
+ 150 mM NaCl

TTBS
25 mM Tris / HCl - buffer pH 7.5
+ 150 mM NaCl
+ 0.05 % Tween 20

PBS + 0.2 mg/ml BSA
20 mM NaH2PO4 buffer pH 7.4
+ 140 mM NaCl
+ 0.2 mg/ml BSA

Antibody solution I:

0.2 µg/ml antibody diluted in 20 ml 1 % low fat milk in TBS

Antibody solution II:

1:5000 dilution
Anti-Mouse-APin 1 % low fat milk in TBS for mouse antibody 4G8 or anti-human-AP in 1 % low fat milk in TBS for humanized anti Aß globulomer antibody 8F5hum8

Dot blot procedure:

1) 1 µl each of the different Aβ-standards (in their 5 serial dilutions) were dotted onto the nitrocellulose membrane in a distance of approximately 1 cm from each other.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6687

2) The Aβ-standards dots were allowed to dry on the nitrocellulose membrane on air for at least 10 min at room temperature (RT) (= dot blot)

3) Blocking:
The dot blot was incubated with 30 ml 5% low fat milk in TBS for 16 h at RT.

4) Washing:
The blocking solution was discarded and the dot blot was incubated under shaking with 20 ml TTBS for 10 min at RT.

5) Antibody solution I:
The washing buffer was discarded and the dot blot was incubated with antibody solution I for 2 h at RT

6) Washing:
The antibody solution I was discarded and the dot blot was incubated under shaking with 20 ml TTBS for 10 min at RT. The washing solution was discarded and the dot blot was incubated under shaking with 20 ml TTBS for 10 min at RT. The washing solution was discarded and the dot blot was incubated under shaking with 20 ml TBS for 10 min at RT.

7) Antibody solution II:
The washing buffer was discarded and the dot blot was incubated with antibody solution II 1h at RT

8) Washing:
The antibody solution II was discarded and the dot blot was incubated under shaking with 20 ml TTBS for 10 min at RT. The washing solution was discarded and the dot blot was incubated under shaking with 20 ml TTBS for 10 min at RT. The washing solution was discarded and the dot blot was incubated under shaking with 20 ml TBS for 10 min at RT.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6688

9) Development:

The washing solution was discarded. The dot blot was developed with a development solution made from 1 tablet of NBT/BCIP (Roche) dissolved in 20mL H_2O for 5 min. The development was stopped by intense washing of the dot blot with H_2O. Quantitative evaluation was done using a densitometric analysis (GS800 densitometer (BioRad) and software package Quantity one, Version 4.5.0 (BioRad)) of the dot-intensity. Only the dots for 10pmol Aß antigen were evaluted.

Results for the discrimination of Aβ monomer against Aβ globulomer by dot blot method: Comparison of 8F5hum8 versus 4G8.

Serial dilutions of Aβ(1-42) globulomer, Aβ1-42 monomer and Aβ1-40 monomer were made in the range from 100pmol/µl-0.01pmol/µl in PBS. Of each sample, 1µl was dotted onto a nitrocellulose membrane. The mouse monoclonal antibody 4G8 (0.2 µg/ml) were used for detection with an anti-mouse IgG coupled to alkaline phosphatase as secondary antibody and the staining reagent NBT/BCIP (Roche Diagnostics, Mannheim). The humanized monoclonal antibody 8F5hum8 (0.2 µg/ml) were used for detection with an anti-human IgG coupled to alkaline phosphatase as secondary antibody and the staining reagent NBT/BCIP (Roche Diagnostics, Mannheim). The detection signal was analyzed in its intensity (reflective density = RD) via a densitometer (GS 800, Biorad, Hercules, CA, USA) at an antigen concentration of 10pmol. At this concentration for every Aβ-form, the measured reflective density was in the linear range of the densitometer detection. The results are shown in the table below:

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6689

	Reflective Density (RD) [10pmol]				
	Aß(1-42) globulomer	Aß(1-42) monomer	Aß(1-40) monomer	Ratio RD Aß(1-42) globulomer / RD Aß(1-42) monomer	Ratio RD Aß(1-42) globulomer / RD Aß(1-40) monomer
8F5 hum8	2.2	1.3	0.04	1.6	59.0
4G8	1.5	3.5	0.15	0.42	10.0

Discrimination of anti-Aβ-antibodies of Aβ1-40 monomer and Aβ1-42 monomer. The discrimination was calculated as the ratio of detection signal of Aβ1-42 globulomer and Aβ1-42 monomer, respectively Aβ1-40 monomer.

In particular, the above results indicate that 8F5hum8 shows a different binding profile compared to commercially available anti-Aβ(1-42) antibody to 4G8, which maps to Aβ (17-24)(i.e., a linear sequence). More specifically, 8F5hum8 show a preference for globulomer binding versus Aβ42 monomer (see column 4 in table with a ratio for Aß(1-42) globulomer / Aß(1-42) monomer of 1.6 for 8F5hum8 versus a ratio of 0.42 for 4G8) as well as a preference for globulomer binding versus Aβ40 (see column 5 in table with a ratio for Aß(1-42) globulomer / Aß(1-40) monomer of 59.0 for 8F5hum8 versus a ratio of 10.0 for 4G8). These two improved binding selectivities over standard 4G8 should result in the production of fewer side effects upon use of 8F5hum8, as described above (e.g., plaque binding).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6690

WHAT IS CLAIMED IS:

1. A binding protein comprising: a) an antigen binding domain which binds to amyloid-beta (1-42) globulomer, said antigen binding domain comprising at least one CDR comprising an amino acid sequence selected from the group consisting of:

CDR-H1. X_1-X_2-X_3-X_4-X_5 (SEQ ID NO:5), wherein;
X_1 is S;
X_2 is Y;
X_3 is G;
X_4 is M; and
X_5 is S.

CDR-H2. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16}-X_{17} (SEQ ID NO:6), wherein;
X_1 is S;
X_2 is I;
X_3 is N;
X_4 is S;
X_5 is N;
X_6 is G;
X_7 is G;
X_8 is S;
X_9 is T;
X_{10} is Y;
X_{11} is Y;
X_{12} is P;
X_{13} is D;
X_{14} is S;
X_{15} is V;
X_{16} is K; and
X_{17} is G.

CDR-H3. X_1-X_2-X_3-X_4 (SEQ ID NO:7), wherein;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6691

X_1 is S;
X_2 is G;
X_3 is D; and
X_4 is Y.

CDR-L1. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9-X_{10}-X_{11}-X_{12}-X_{13}-X_{14}-X_{15}-X_{16} (SEQ ID NO:8), wherein:
X_1 is R;
X_2 is S;
X_3 is S;
X_4 is Q;
X_5 is S;
X_6 is L;
X_7 is V;
X_8 is Y;
X_9 is S;
X_{10} is N;
X_{11} is G;
X_{12} is D;
X_{13} is T;
X_{14} is Y;
X_{15} is L; and
X_{16} is H.

CDR-L2. X_1-X_2-X_3-X_4-X_5-X_6-X_7 (SEQ ID NO:9), wherein;
X_1 is K;
X_2 is V;
X_3 is S;
X_4 is N;
X_5 is R;
X_6 is F; and
X_7 is R.

and

CDR-L3. X_1-X_2-X_3-X_4-X_5-X_6-X_7-X_8-X_9 (SEQ ID NO:10), wherein:
X_1 is S;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6692

X_2 is Q;
X_3 is S;
X_4 is T;
X_5 is H;
X_6 is V;
X_7 is P;
X_8 is W; and
X_9 is T,

and 2) a human acceptor framework comprising at least one acceptor sequence selected from the group consisting of SEQ ID NO.:17, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, SEQ ID NO.:22, SEQ ID NO.:23 and SEQ ID NO.:24.

2. The binding protein according to claim 1, wherein said at least one CDR comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO.:11, SEQ ID NO.:12, SEQ ID NO.:13, SEQ ID NO.:14, SEQ ID NO.:15, and SEQ ID NO.:16.

3. The binding protein according to claim 1, wherein said binding protein comprises at least 3 CDRs.

4. The binding protein according to claim 3, wherein said at least 3 CDRs are selected from a variable domain CDR set consisting of:

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
1	**VH 8F5 (hum8)**		EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLELVASINSNGGSTYYPDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCASGDYWGQGTLVTVSS

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6693

SEQ ID No.	Protein region		Sequence
			123456789**0**123456789**0**123456789**0**
	VH 8F5 (hum8) CDR-H1	Residues 31-35 of SEQ ID NO.:1	**SYGMS (SEQ ID NO:11)**
	VH 8F5 (hum8) CDR-H2	Residues 50-66 of SEQ ID NO.:1	**SINSNGGSTYYPDSVKG (SEQ ID NO:12)**
	VH 8F5 (hum8) CDR-H3	Residues 98-108 of SEQ ID NO.:1	**SGDY (SEQ ID NO:13)**
2	**VL 8F5 (hum8)**		DIVMTQSPLSLPVTPGEPASISCRSSQSLVYSNGDTYLHWYLQKPGQSPKLLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPWTFGGGTKVEIKR
	VL 8F5 (hum8) CDR-L1	Residues 24-39 of SEQ ID NO.:2	**RSSQSLVYSNGDTYLH (SEQ ID NO:14)**
	VL 8F5 (hum8) CDR-L2	Residues 55-61 of SEQ ID NO.:2	**KVSNRFS (SEQ ID NO:15)**
	VL 8F5 (hum8) CDR-L3	Residues 94-102 of SEQ ID NO.:2	**SQSTHVPWT (SEQ ID NO:16)**

5. The binding protein according to claim 4, two variable domain CDR sets.

6. The binding protein according to claim 5, wherein said two variable domain CDR sets are VH 8F5 CDR Set & VL 8F5 CDR Set.

7. The binding protein according to claim 3, further comprising a human acceptor framework.

8. The binding protein according to claim 4, further comprising a human acceptor framework.

9. The binding protein according to claim 5, further comprising a human acceptor framework.

10. The binding protein according to claim 6, further comprising a human acceptor framework.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6694

11. The binding protein according to claim 7, wherein said human acceptor framework comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO.:17, SEQ ID NO.: 18, SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, SEQ ID NO.:22, SEQ ID NO.:23 and SEQ ID NO.:24.

12. The binding protein according to claim 8, wherein said human acceptor framework comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO.:17, SEQ ID NO.: 18, SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, SEQ ID NO.:22, SEQ ID NO.:23 and SEQ ID NO.:24.

13. The binding protein according to claim 9, wherein said human acceptor framework comprises an amino acid sequence selected from the group consisting of:

SEQ ID NO.:17, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, SEQ ID NO.:22, SEQ ID NO.:23 and SEQ ID NO.:24.

14. The binding protein according to claim 10, wherein said human acceptor framework comprises amino acid sequence selected from the group consisting of:

SEQ ID NO.:17, SEQ ID NO.:18, SEQ ID NO.:19, SEQ ID NO.:20, SEQ ID NO.:21, SEQ ID NO.:22, SEQ ID NO.:23 and SEQ ID NO.:24.

15. The binding protein according to claim 1, wherein said binding protein comprises at least one variable domain

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6695

having an amino acid sequence selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2.

16. The binding protein according to claim 15 wherein said binding protein comprises two variable domains, wherein said two variable domains have amino acid sequences selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2.

17. The binding protein according to claim 7, wherein said human acceptor framework comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with Aß(1-42) globulomer;
a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone; and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

18. The binding protein according to claim 10, wherein said human acceptor framework comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with an Aß(1-42) globulomer;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6696

a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone;and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

19. The binding protein according to claim 16, wherein said human acceptor framework comprises at least one Framework Region amino acid substitution at a key residue, said key residue selected from the group consisting of:
a residue adjacent to a CDR;
a glycosylation site residue;
a rare residue;
a residue capable of interacting with an Aß(1-42) globulomer;
a residue capable of interacting with a CDR;
a canonical residue;
a contact residue between heavy chain variable region and light chain variable region;
a residue within a Vernier zone;and
a residue in a region that overlaps between a Chothia-defined variable heavy chain CDR1 and a Kabat-defined first heavy chain framework.

20. The binding protein according to claim 7, wherein said human acceptor framework comprises at least one Framework Region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprises at least 52 amino acid residues identical to said human acceptor framework.

21. The binding protein according to claim 10, wherein

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6697

said human acceptor framework comprises at least one Framework Region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprises at least 52 amino acid residues identical to said human acceptor framework.

22. The binding protein according to claim 16, wherein said human acceptor framework comprises at least one Framework Region amino acid substitution, wherein the amino acid sequence of the framework is at least 65% identical to the sequence of said human acceptor framework and comprises at least 52 amino acid residues identical to said human acceptor framework.

23. The binding protein according to claim 1, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2 and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

24. The binding protein according to claim 23 wherein said binding protein comprises two variable domains, wherein said two variable domains have amino acid sequences of: SEQ ID NO.:1 and SEQ ID NO.:2.

25. The binding protein according to claim 20, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2 and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6698

26. The binding protein according to claim 21, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2.

27. The binding protein according to claim 22, wherein said binding protein comprises at least one variable domain having an amino acid sequence selected from the group consisting of: SEQ ID NO.:1 and SEQ ID NO.:2.

28. The binding protein according to claim 1, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

29. The binding protein according to claim 4, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

30. The binding protein according to claim 6, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

31. The binding protein according to claim 7, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

32. The binding protein according to claim 11, wherein the binding protein binds Aß(1-42) globulomer and said

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6699

binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

33. The binding protein according to claim 15, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

34. The binding protein according to claim 17, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

35. The binding protein according to claim 20, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

36. The binding protein according to claim 23, wherein the binding protein binds Aß(1-42) globulomer and said binding protein preferentially binds to the soluble form of Aß(1-42) globulomer as compared to the fibrillar form.

37. The binding protein according to claim 28, wherein the binding protein modulates a biological function of Aß(1-42) globulomer.

38. The binding protein according to claim 33, wherein the binding protein modulates a biological function of Aß(1-42) globulomer.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6700

39. The binding protein according to claim 36, wherein the binding protein modulates a biological function of Aß(1-42) globulomer.

40. The binding protein according to claim 28, wherein the binding protein neutralizes Aß(1-42) globulomer.

41. The binding protein according to claim 33, wherein the binding protein neutralizes Aß(1-42) globulomer.

42. The binding protein according to claim 36, wherein the binding protein neutralizes Aß(1-42) globulomer.

43. The binding protein according to claim 28, wherein said binding protein has a dissociation constant (K_D) to said target selected from the group consisting of: at most about 10^{-7} M, at most about 10^{-8} M, at most about 10^{-9} M, at most about 10^{-10} M, at most about 10^{-11} M, at most about 10^{-12} M, and at most about 10^{-13} M.

44. The binding protein according to claim 33, wherein said binding protein has a dissociation constant (K_D) to said target selected from the group consisting of: at most about 10^{-7} M, at most about 10^{-8} M, at most about 10^{-9} M, at most about 10^{-10} M, at most about 10^{-11} M, at most about 10^{-12} M, and at most about 10^{-13} M.

45. The binding protein according to claim 35, wherein said binding protein has a dissociation constant (K_D) to said target selected from the group consisting of: at most about 10^{-7} M, at most about 10^{-8} M, at most about 10^{-9} M, at most about 10^{-10} M, at most about 10^{-11} M, at most about 10^{-12} M, and at most about 10^{-13} M.

46. The binding protein according to claim 36, wherein said binding protein has a dissociation constant (K_D) to said

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6701

target selected from the group consisting of: at most about 10^{-7} M, at most about 10^{-8} M, at most about 10^{-9} M, at most about 10^{-10} M, at most about 10^{-11} M, at most about 10^{-12} M, and at most about 10^{-13} M.

47. An antibody construct comprising said binding protein of any one of claims 1-46, said antibody construct further comprising a linker polypeptide or an immunoglobulin constant domain.

48. The antibody construct according to claim 47, wherein said binding protein is selected from the group consisting of:

an immunoglobulin molecule,
a monoclonal antibody,
a chimeric antibody,
a CDR-grafted antibody,
a humanized antibody,
a Fab,
a Fab',
a F(ab')2,
a Fv,
a disulfide linked Fv,
a scFv,
a single domain antibody,
a diabody,
a multispecific antibody,
a dual specific antibody,
a isotype antibody,
a mutated antibody, a dual variable domain antibody and
a bispecific antibody.

49. The antibody construct according to claim 47, wherein said binding protein comprises a heavy chain

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6702

immunoglobulin constant domain selected from the group consisting of:
a human IgM constant domain,
a human IgG1 constant domain,
a human IgG2 constant domain,
a human IgG3 constant domain,
domain,
a human IgG4 constant domain,
a human IgE constant domain,
and
a human IgA constant domain.

50. The antibody construct according to claim 47, comprising an immunnoglobulin constant domain having an amino acid sequence selected from the group consisting of: SEQ ID NO.:25, SEQ ID NO.:26, SEQ ID NO.:27 and SEQ ID NO.:28.

51. An antibody conjugate comprising an antibody construct described in any one of claims 47-50, said antibody conjugate further comprising an agent selected from the group consisting of: an immunoadhension molecule, an imaging agent, a therapeutic agent, and a cytotoxic agent.

52. The antibody conjugate according to claim 51, wherein said agent is an imaging agent selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, and biotin.

53. The antibody conjugate according to claim 52, wherein said radiolabel is selected from the group consisting of: ^{3}H, ^{14}C, ^{35}S, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I, ^{177}Lu, ^{166}Ho, and ^{153}Sm.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6703

54. The antibody conjugate according to claim 51, wherein said agent is a therapeutic or cytotoxic agent selected from the group consisting of: an anti-metabolite, an alkylating agent, an antibiotic, a growth factor, a cytokine, an anti-angiogenic agent, an anti-mitotic agent, an anthracycline, toxin, and an apoptotic agent.

55. The antibody construct according to claim 49, wherein said binding protein possesses a human glycosylation pattern.

56. The antibody conjugate according to claim 51, wherein said binding protein possesses a human glycosylation pattern.

57. The binding protein according to claim 3, wherein said binding protein exists as a crystal.

58. The antibody construct according to claim 47, wherein said antibody construct exists as a crystal.

59. The antibody conjugate according to claim 51, wherein said antibody construct exists as a crystal.

60. The binding protein according to claim 57, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

61. The antibody construct according to claim 58, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

62. The antibody conjugate according to claim 59, wherein said crystal is a carrier-free pharmaceutical controlled release crystal.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6704

63. The binding protein according to claim 57, wherein said binding protein has a greater half life in vivo than the soluble counterpart of said binding protein.

64. The antibody construct according to claim 58, wherein said antibody construct has a greater half life in vivo than the soluble counterpart of said antibody construct.

65. The antibody conjugate according to claim 59, wherein said antibody conjugate has a greater half life in vivo than the soluble counterpart of said antibody conjugate.

66. The binding protein according to claim 57, wherein said binding protein retains biological activity.

67. The antibody construct according to claim 58, wherein said antibody construct retains biological activity.

68. The antibody conjugate according to claim 59, wherein said antibody conjugate retains biological activity.

69. An isolated nucleic acid molecule encoding a binding protein, wherein the amino acid sequence of said variable heavy chain of said binding protein has at least 70% identity to SEQ ID NO:1.

70. An isolated nucleic acid molecule encoding a binding protein, wherein the amino acid sequence of said variable light chain of said binding protein has at least 70% identity to SEQ ID NO:2.

71. An isolated nucleic acid molecule encoding a binding protein amino acid sequence of any one of claims 1-46.

72. An isolated nucleic acid molecule encoding an antibody contruct amino acid sequence of any one of claims 47-50.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6705

73. An isolated nucleic acid molecule encoding an antibody conjugate amino acid sequence of any one of claims 51-53.

74. A vector comprising said nucleic acid molecule of any one of claims 71-73.

75. A host cell comprising said vector of claim 74.

76. The host cell of claim 75, wherein said host cell is a prokaryotic cell.

77. The host cell according to claim 76, wherein said host cell is *Escherichia coli*.

78. The host cell according to claim 75, wherein said host cell is a eukaryotic cell.

79. The host cell according to claim 78, wherein said eukaryotic cell is selected from the group consisting of protist cell, animal cell, plant cell and fungal cell.

80. The host cell according to claim 79, wherein said animal cell is selected from the group consisting of a mammalian cell, an avian cell, and an insect cell.

81. The host cell according to claim 80, wherein said mammalian cell is a CHO cell.

82. The host cell according to claim 80, wherein said host cell is COS.

83. The host cell according to claim 80, wherein said fungal cell is a yeast cell.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6706

84. The host cell according to claim 83, wherein said yeast cell is *Saccharomyces cerevisiae*.

85. The host cell according to claim 80, wherein said insect cell is Sf9.

86. A method of producing a protein capable of binding Aß(1-42) globulomer, comprising culturing a host cell of any one of claims 75-85 for a time and under conditions sufficient to produce a binding protein capable of binding Aß(1-42) globulomer.

87. An isolated protein produced according to the method of claim 86.

88. A composition for the release of a binding protein said composition comprising:
(a) a formulation, wherein said formulation comprises a crystallized binding protein, according to any one of claims 57-68, and an ingredient; and
(b) at least one polymeric carrier.

89. The composition according to claim 88, wherein said polymeric carrier is at least one polymer selected the group consisting of: poly (acrylic acid), poly (cyanoacrylates), poly (amino acids), poly (anhydrides), poly (depsipeptide), poly (esters), poly (lactic acid), poly (lactic-co-glycolic acid) or PLGA, poly (b-hydroxybutryate), poly (caprolactone), poly (dioxanone); poly (ethylene glycol), poly ((hydroxypropyl) methacrylamide, poly [(organo) phosphazene], poly (ortho esters), poly (vinyl alcohol), poly (vinylpyrrolidone), maleic anhydride-alkyl vinyl ether copolymers, pluronic polyols, albumin, alginate, cellulose and cellulose derivatives, collagen, fibrin, gelatin, hyaluronic acid,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6707

oligosaccharides, glycaminoglycans, sulfated polyeaccharides, blends and copolymers thereof.

90. The composition according to claim 87, wherein said ingredient is selected from the group consisting of albumin, sucrose, trehalose, lactitol, gelatin, hydroxypropyl-cyclodextrin, methoxypolyethylene glycol and polyethylene glycol.

91. A method for treating a mammal suspected of having an amyloidosis comprising administering to the mammal said composition of claim 88 in an amount sufficient to effect said treatment.

92. A pharmaceutical composition comprising the binding protein of claim 1, and a pharmaceutically acceptable carrier.

93. The pharmaceutical composition of claim 91 wherein said pharmaceutically acceptable carrier functions as adjuvant useful to increase the absorption, or dispersion of said binding protein.

94. The pharmaceutical composition of claim 92 wherein said adjuvant is hyaluronidase.

95. The pharmaceutical composition of claim 91 further comprising at least one additional therapeutic agent for treating a disorder in which presence of Aß(1-42) globulomer is detrimental.

96. The method of claim 95 wherein said at least one additional therapeutic agent is selected from the group consisting of a cholesterinase inhibitor, a TNF antagonist, a cytokine antagonist, a partial NMDA receptor blocker, a glycosaminoglycan mimetic, an inhibitor or allosteric modulator of gamma secretase, a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6708

luteinizing hormone blockade gonadotropin releasing hormone agonist, a serotinin 5-HT1A receptor antagonist, a chelating agent, a neuronal selective L-type calcium channel blocker, an immunomodulator, an amyloid fibrillogenesis inhibitor or amyloid protein deposition inhibitor, a PDE4 inhibitor, a histamine agonist, a receptor protein for advanced glycation end products, a PARP stimulator, a serotonin 6-receptor antagonist, a 5-HT4 receptor agonist, a human steroid, a glucose uptake stimulant which enhanceds neuronal metabolism, a selective CB1 antagonist, a partial agonist at benzodiazepine receptors, an amyloid beta production antagonist or inhibitor, an amyloid beta deposition inhibitor, a NNR alpha-7 partial antagonist, a therapeutic targeting PDE4, a RNA translation inhibitor, a muscarinic agonist, a nerve growth factor receptor agonist, a NGF receptor agonist and a gene therapy modulator.

97. A method for reducing Aß(1-42) globulomer activity comprising contacting Aß(1-42) globulomer with the binding protein of claim 1 such that Aß(1-42) globulomer activity is reduced.

98. A method for reducing human Aß(1-42) globulomer activity in a human subject suffering from a disorder in which Aß(1-42) globulomer is detrimental, comprising administering to the human subject the binding protein of claim 1 such that human Aß(1-42) globulomer activity in the human subject is reduced.

99. A method for treating a subject for a disease or a disorder in which Aß(1-42) globulomer activity is detrimental by administering to the subject the binding protein of claim 1 in an amount sufficient to effect said treatment.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6709

100. The method of claim 99, wherein said disease or disorder is selected from the group consisting of Alpha1-antitrypsin-deficiency, C1-inhibitor deficiency angioedema, Antithrombin deficiency thromboembolic disease, Kuru, Creutzfeld-Jacob disease/scrapie, Bovine spongiform encephalopathy, Gerstmann-Straussler-Scheinker disease, Fatal familial insomnia, Huntington's disease, Spinocerebellar ataxia, Machado-Joseph atrophy, Dentato-rubro-pallidoluysian atrophy, Frontotemporal dementia, Sickle cell anemia, Unstable hemoglobin inclusion-body hemolysis, Drug-induced inclusion body hemolysis, Parkinson's disease, Systemic AL amyloidosis, Nodular AL amyloidosis, Systemic AA amyloidosis, Prostatic amyloid, Hemodialysis amyloidosis, Hereditary (Icelandic) cerebral angiopathy, Huntington's disease, Familial visceral amyloid, Familial visceral polyneuropathy, Familial visceral amyloidosis, Senile systemic amyloidosis, Familial amyloid neurophathy, Familial cardiac amyloid, Alzheimer's disease, Down's syndrome, Medullary carcinoma thyroid and Type 2 diabetes mellitus (T2DM).

101. A method of treating a patient suffering from a disorder in which Aß(1-42) globulomer is detrimental comprising the step of administering the binding protein of claim 1 before, concurrent, or after the administration of at least one second agent, wherein said at least one second agent is selected from the group consisting of a cholesterinase inhibitor, a partial NMDA receptor blocker, a glycosaminoglycan mimetic, a TNF antagonist, a cytokine antagonist, an inhibitor or allosteric modulator of gamma secretase, a luteinizing hormone blockade gonadotropin releasing hormone agonist, a serotinin 5-HT1A receptor antagonist, a chelating agent, a neuronal selective L-

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6710

type calcium channel blocker, an immunomodulator, an amyloid fibrillogenesis inhibitor or amyloid protein deposition inhibitor, a PDE4 inhibitor, a histamine agonist, a receptor protein for advanced glycation end products, a PARP stimulator, a serotonin 6-receptor antagonist, a 5-HT4 receptor agonist, a human steroid, a glucose uptake stimulant which enhances neuronal metabolism, a selective CB1 antagonist, a partial agonist at benzodiazepine receptors, an amyloid beta production antagonist or inhibitor, an amyloid beta deposition inhibitor, a NNR alpha-7 partial antagonist, a therapeutic targeting PDE4, a RNA translation inhibitor, a muscarinic agonist, a nerve growth factor receptor agonist, a NGF receptor agonist and a gene therapy modulator.

102. The method of claim 101 wherein said cholesterinase inhibitor is selected from the group consisting of Tacrine, Donepezil, Rivastigmine and Galantamine.

103. The method of claim 101 wherein said partial NMDA receptor blocker is Memantine.

104. The method according to claim 98, wherein said administering to the subject is by at least one mode selected from the group consisting of parenteral, subcutaneous, intramuscular, intravenous, intrarticular, intrabronchial, intraabdominal, intracapsular, intracartilaginous, intracavitary, intracelial, intracerebellar, intracerebroventricular, intracolic, intracervical, intragastric, intrahepatic, intramyocardial, intraosteal, intrapelvic, intrapericardiac, intraperitoneal, intrapleural, intraprostatic, intrapulmonary, intrarectal, intrarenal, intraretinal, intraspinal, intrasynovial, intrathoracic, intrauterine, intravesical, bolus,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6711

vaginal, rectal, buccal, sublingual, intranasal, and transdermal.

105. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with said binding protein of claim 1 for a time and under conditions sufficient for formation of globulomer/binding protein complexes; and

c. detecting presence of said globulomer/binding protein complexes in said sample, presence of said complexes indicating a diagnosis of Alzheimer's Disease in said patient.

106. A method of diagnosing Alzheimer's Disease in a patient suspected of having this disease comprising the steps of:

a. isolating a biological sample from said patient;

b. contacting said biological sample with said binding protein of claim 1 for a time and under conditions sufficient for the formation of globulomer/binding protein complexes;

c. adding a conjugate to the resulting globulomer/binding protein complexes for a time and under conditions sufficient to allow said conjugate to bind to the bound binding protein, wherein said conjugate comprises an anti-binding protein antibody attached to a signal generating compound capable of generating a detectable signal; and

d. detecting the presence of said binding protein which may be present in said biological sample by detecting a signal generated by said signal generating

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6712

compound, said signal indicating a diagnosis of Alzheimer's Disease in said patient.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6713

FIGURE 1

8F5 VH (hum8)

FIGURE 1(A) – SEQ ID NO.: 3

GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTAGTGCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGATTCACTTTCAGTAGCTATGGCATGTCT
TGGGTTCGCCAGGCTCCAGGCAAGGGGCTGGAATTGGTCGCAAGCATCAATA
GTAATGGTGGTAGCACCTATTATCCAGACAGTGTGAAGGGCCGATTCACCAT
CTCCAGAGACAATGCCAAGAACACCCTGTACCTGCAAATGAACAGTCTGAGG
GCTGAGGACACAGCCGTGTATTACTGTGCAAGTGGTGACTACTGGGGCCAAG
GCACCCTTGTCACAGTCTCCTCA

FIGURE 1(B) – SEQ ID NO.: 1

EVQLVESGGGLVQPGGSLRLSCAASGFTFSSYGMSWVRQAPGKGLELVASINSN
GGSTYYPDSVKGRFTISRDNAKNTLYLQMNSLRAEDTAVYYCASGDYWGQGTL
VTVSS

****

8F5 VL (hum8)

FIGURE 1(C) – SEQ ID NO.: 4

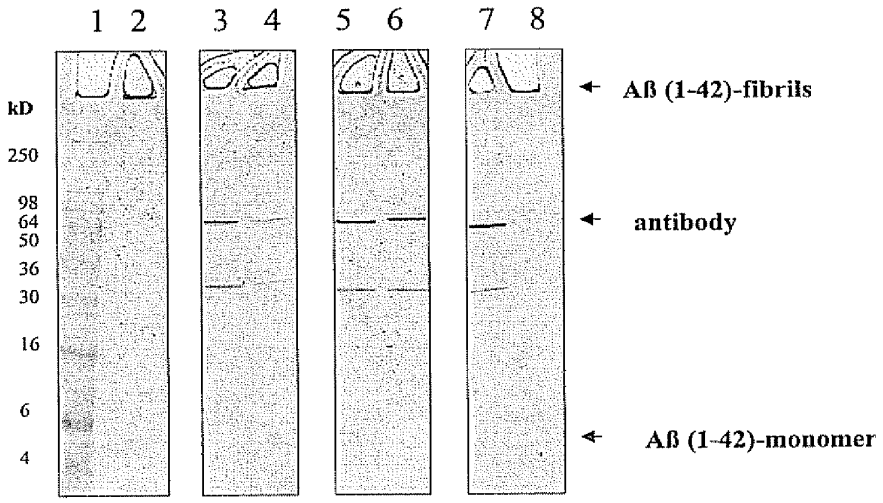
GATATTGTGATGACCCAATCTCCACTCTCCCTGCCTGTCACTCCTGGAGAACC
AGCCTCCATCTCTTGCAGATCTAGTCAGAGCCTTGTATATAGTAATGGAGACA
CCTATTTACATTGGTACCTGCAGAAGCCAGGCCAGTCTCCAAAGCTCCTGATC
TACAAAGTTTCCAACCGATTTTCTGGGGTCCCAGACAGGTTCAGTGGCAGTG
GATCAGGGACAGATTTCACACTCAAGATCAGCAGAGTGGAGGCTGAGGATGT
GGGAGTTTATTACTGCTCTCAAAGTACACATGTTCCTTGGACGTTCGGTGGAG
GCACCAAGGTAGAAATCAAACGG

FIGURE 1(D) – SEQ ID NO.: 2

DIVMTQSPLSLPVTPGEPASISCRSSQSLVYSNGDTYLHWYLQKPGQSPKLLIYKV
SNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPWTFGGGTKVEI
KR

FIGURE 2

A



B

	antibody bound to fibrils [%]
6E10	91
8F5hum8	20
IgG2a	9

FIGURE 3

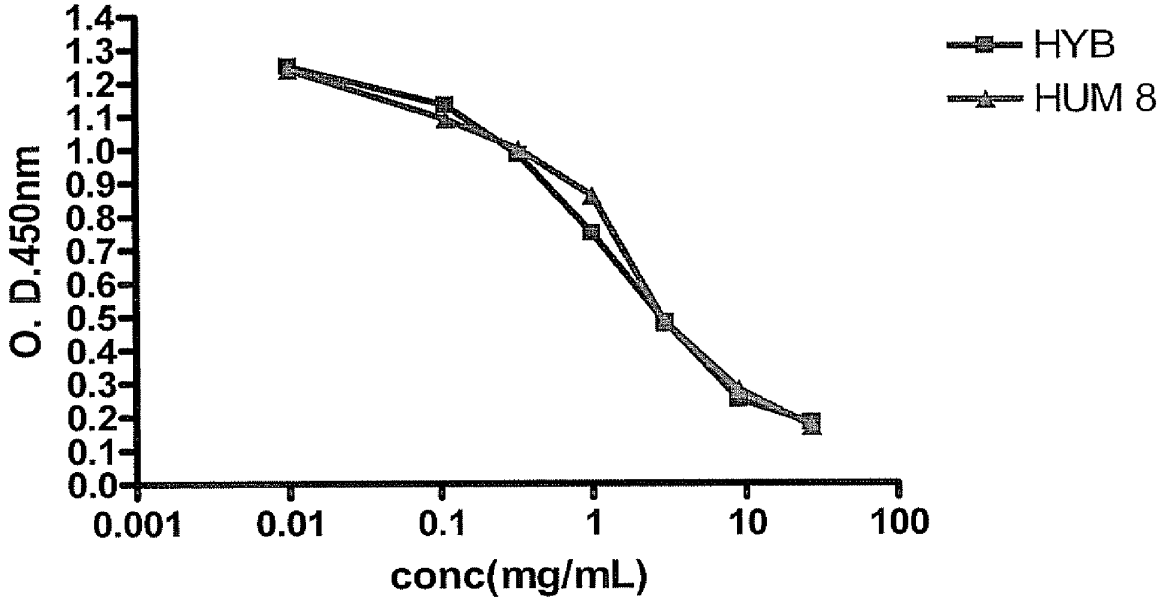

1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
O. D.450nm
0.001
0.01
0.1
1
10
100
conc(mg/mL)
HYB
HUM 8

FIGURE 4

```
8F5VH          E V Q L V E S G G G L V Q P G G S L K L S C A A S G F T F S
Hu8F5VHv1      E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
Hu8F5VHv2      E V Q L V E S G G G L V Q P G G S L R L S C A A S G F T F S
YSE'CL         E V Q L V E S G G G L V K P G G S L R L S C A A S G F T F S

                                                                      60
8F5VH          S Y G M S W V R Q T P D K R L E L V A S I N S N G G S T Y Y
Hu8F5VHv1      S Y G M S W V R Q A P G K G L E W V A S I N S N G G S T Y Y
Hu8F5VHv2      S Y G M S W V R Q A P G K G L E L V A S I N S N G G S T Y Y
YSE'CL         - - - - - W V R Q A P G K G L E W V S - - - - - - - - - -

                                                                      90
8F5VH          P D S V K G R F T I S R D N A K N T L Y L Q M S S L K S E D
Hu8F5VHv1      P D S V K G R F T I S R D N A K N T L Y L Q M N S L R A E D
Hu8F5VHv2      P D S V K G R F T I S R D N A K N T L Y L Q M N S L R A E D
YSE'CL         - - - - - - R F T I S R D N A K N S L Y L Q M N S L R A E D

                                                                     112
8F5VH          T A M Y Y C A S G D Y W G Q G S T L T V S S
Hu8F5VHv1      T A V Y Y C A S G D Y W G Q G T L V T V S S
Hu8F5VHv2      T A V Y Y C A S G D Y W G Q G T L V T V S S
YSE'CL/JH4     T A V Y Y C A R - - - W G R G T L V T V S S
```

FIGURE 5

```
                                                                       30
8F5VL        D V V M T Q T P L S L P V S L G D Q A S I S C R S S Q S L V
Hu8F5VL      D I V M T Q S P L S L P V T P G E P A S I S C R S S Q S L V
TR1.37'CL    E L V M T Q S P L S L P V T P G E P A S I S C - - - - - - -

                                                                       60
8F5VL        Y S N G D T Y L H W Y L Q K P G Q S P K L L I Y K V S N R F
Hu8F5VL      Y S N G D T Y L H W Y L Q K P G Q S P K L L I Y K V S N R F
TR1.37'CL    - - - - - - - - - W Y L Q K P G Q S P Q L L I Y - - - - - -

                                                                       90
8F5VL        S G V P D R F S G S G S G T D F T L K I S R V E A E D L G V
Hu8F5VL      S G V P D R F S G S G S G T D F T L K I S R V E A E D V G V
TR1.37'CL    - G V P D R F S G S G S G T D F T L K I S R V E A E D V G V

                                                 112
8F5VL        Y F C S Q S T H V P W T F G G G T K L E I K
Hu8F5VL      Y Y C S Q S T H V P W T F G G G T K V E I K
TR1.37'CL/   Y Y C - - - - - - - - - F G G G T K V E I K
JK4
```

```
                                30                                  60
ACGCGTCCACCATGGAGTTCGGCCTGAGCTGGCTGTTCCTGGTGGCCATCCTGAAGGGCG
            M  E  F  G  L  S  W  L  F  L  V  A  I  L  K  G

                                90                                 120
TGCAGTGCGAGGTGCAGCTGGTGGAGAGCGGCGGCGGCCTGGTGCAGCCTGGCGGCAGCC
V  Q  C  E  V  Q  L  V  E  S  G  G  G  L  V  Q  P  G  G  S

                               150                                 180
TGCGCCTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGGCATGAGCTGGGTGC
L  R  L  S  C  A  A  S  G  F  T  F  S  S  Y  G  M  S  W  V

                               210                                 240
GCCAGGCCCCTGGCAAGGGCCTGGAGTGGGTGGCCAGCATCAACAGCAACGGCGGCAGCA
R  Q  A  P  G  K  G  L  E  W  V  A  S  I  N  S  N  G  G  S

                               270                                 300
CCTACTACCCTGACAGCGTGAAGGGCCGCTTCACCATCAGCCGCGACAACGCCAAGAACA
T  Y  Y  P  D  S  V  K  G  R  F  T  I  S  R  D  N  A  K  N

                               330                                 360
CCCTGTACCTGCAGATGAACAGCCTGCGCGCCGAGGACACCGCCGTGTACTACTGCGCCA
T  L  Y  L  Q  M  N  S  L  R  A  E  D  T  A  V  Y  Y  C  A

                               390                                 420
GCGGCGACTACTGGGGCCAGGGCACCCTCGTCACCGTCTCCTCAGGTGAGTCCTCACAAC
S  G  D  Y  W  G  Q  G  T  L  V  T  V  S  S

    427
CTCTAGA
```

FIGURE 6

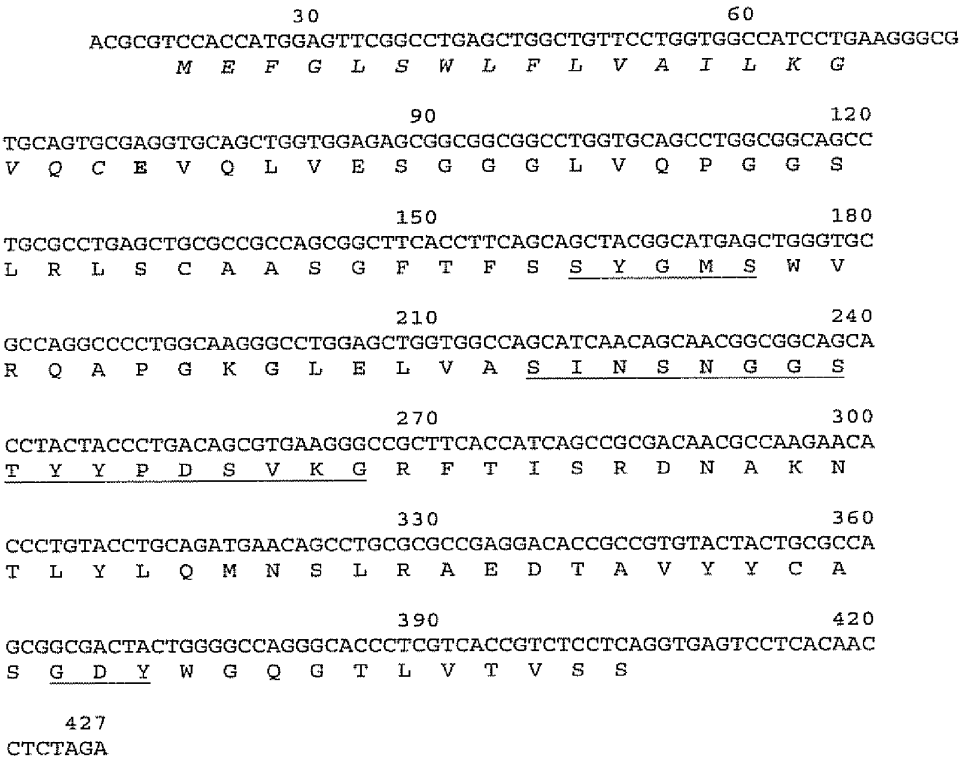

30
60
ACGCGTCCACCATGGAGTTCGGCCTGAGCTGGCTGTTCCTGGTGGCCATCCTGAAGGGCG
M E F G L S W L F L V A I L K G
90
120
TGCAGTGCGAGGTGCAGCTGGTGGAGAGCGGCGGCGGCCTGGTGCAGCCTGGCGGCAGCC
V Q C E V Q L V E S G G G L V Q P G G S
150
180
TGCGCCTGAGCTGCGCCGCCAGCGGCTTCACCTTCAGCAGCTACGGCATGAGCTGGGTGC
L R L S C A A S G F T F S S Y G M S W V
210
240
GCCAGGCCCCTGGCAAGGGCCTGGAGCTGGTGGCCAGCATCAACAGCAACGGCGGCAGCA
R Q A P G K G L E L V A S I N S N G G S
270
300
CCTACTACCCTGACAGCGTGAAGGGCCGCTTCACCATCAGCCGCGACAACGCCAAGAACA
T Y Y P D S V K G R F T I S R D N A K N
330
360
CCCTGTACCTGCAGATGAACAGCCTGCGCGCCGAGGACACCGCCGTGTACTACTGCGCCA
T L Y L Q M N S L R A E D T A V Y Y C A
390
420
GCGGCGACTACTGGGGCCAGGGCACCCTCGTCACCGTCTCCTCAGGTGAGTCCTCACAAC
S G D Y W G Q G T L V T V S S
427
CTCTAGA

FIGURE 7

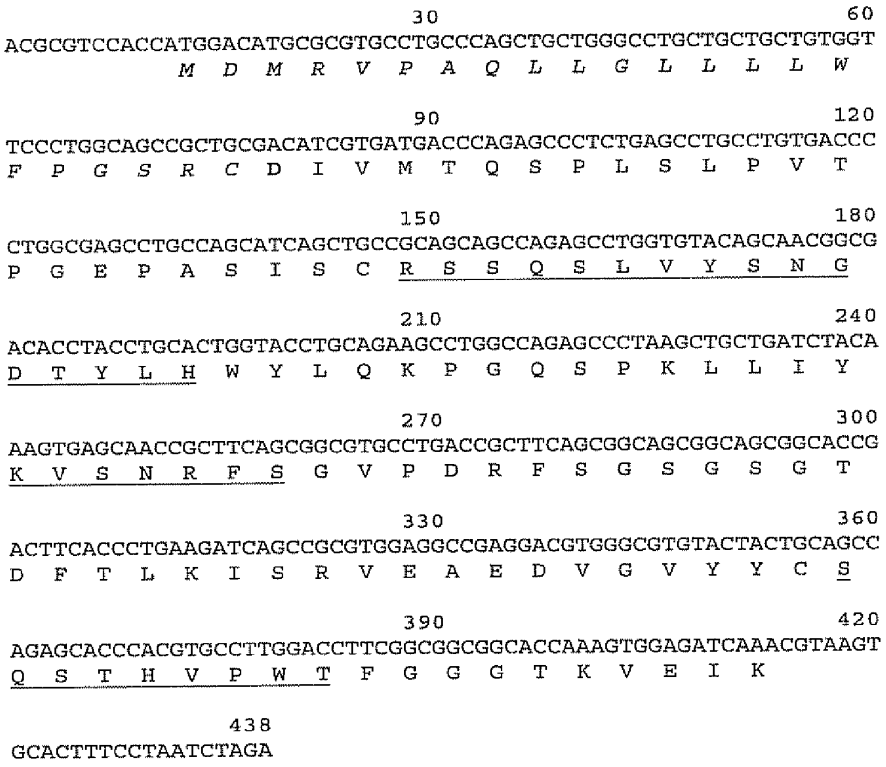
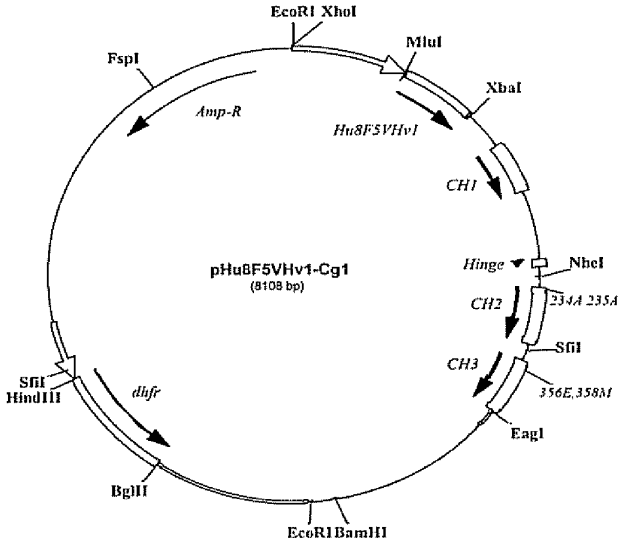

30 60
ACGCGTCCACCATGGACATGCGCGTGCCTGCCCAGCTGCTGGGCCTGCTGCTGCTGTGGT
M D M R V P A Q L L G L L L L W
90 120
TCCCTGGCAGCCGCTGCGACATCGTGATGACCCAGAGCCCTCTGAGCCTGCCTGTGACCC
F P G S R C D I V M T Q S P L S L P V T
150 180
CTGGCGAGCCTGCCAGCATCAGCTGCCGCAGCAGCCAGAGCCTGGTGTACAGCAACGGCG
P G E P A S I S C R S S Q S L V Y S N G
210 240
ACACCTACCTGCACTGGTACCTGCAGAAGCCTGGCCAGAGCCCTAAGCTGCTGATCTACA
D T Y L H W Y L Q K P G Q S P K L L I Y
270 300
AAGTGAGCAACCGCTTCAGCGGCGTGCCTGACCGCTTCAGCGGCAGCGGCAGCGGCACCG
K V S N R F S G V P D R F S G S G S G T
330 360
ACTTCACCCTGAAGATCAGCCGCGTGGAGGCCGAGGACGTGGGCGTGTACTACTGCAGCC
D F T L K I S R V E A E D V G V Y Y C S
390 420
AGAGCACCCACGTGCCTTGGACCTTCGGCGGCGGCACCAAAGTGGAGATCAAACGTAAGT
Q S T H V P W T F G G G T K V E I K
438
GCACTTTCCTAATCTAGA

FIGURE 8


EcoRI XhoI
MluI
FspI
XbaI
Amp-R
Hu8F5VHv1
CH1
pHu8F5VHv1-Cg1
(8108 bp)
Hinge
NheI
CH2
234A 235A
SfiI
CH3
356E,358M
SfiI
HindIII
dhfr
EagI
BglII
EcoRI BamHI

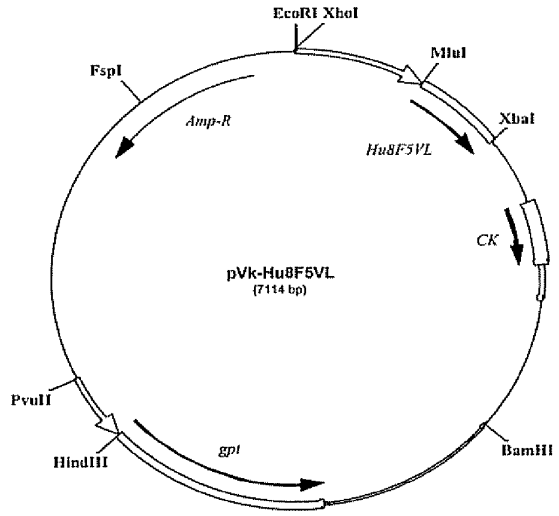

EcoRI XhoI
MluI
FspI
Amp-R
XbaI
Hu8F5VL
CK
pVk-Hu8F5VL
(7114 bp)
PvuII
HindIII
gpt
BamHI

FIGURE 9

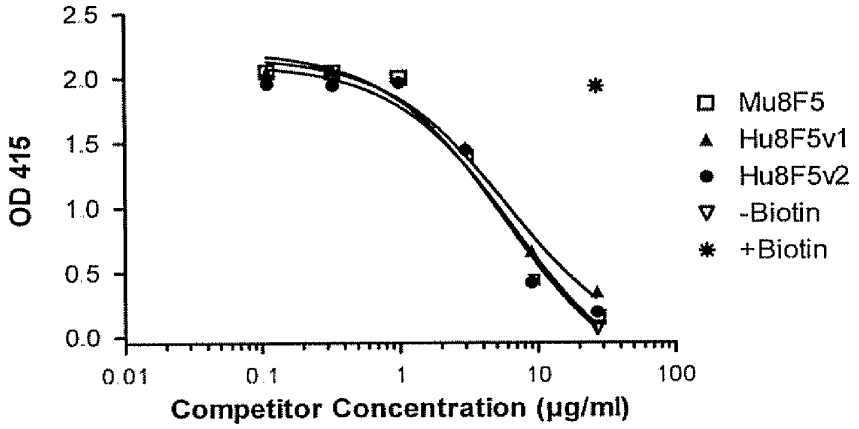

2.5
2.0
1.5
1.0
0.5
0.0
OD 415
0.01
0.1
1
10
100
Competitor Concentration (µg/ml)
Mu8F5
Hu8F5v1
Hu8F5v2
-Biotin
+Biotin

FIGURE 10

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/065199

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/18

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2006/094724 A (ABBOTT GMBH & CO KG [DE]; HILLEN HEINZ [DE]; STRIEBINGER ANDREAS [DE];) 14 September 2006 (2006-09-14) examples 1-6,8,9,14 figure 12a ----- -/--	1-106

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
12 September 2008	26/09/2008

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Bumb, Peter

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/065199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 03/090772 A (LILLY CO ELI [US]; GERLAI ROBERT THOMAS [US]) 6 November 2003 (2003-11-06) sequences 9-10	1-14,17, 18,20, 21,23, 28-32, 34-37, 39,40, 42,43, 45-50, 69-72, 74,75, 86,87, 91,92, 97-100, 104
X	WO 01/62801 A (UNIV WASHINGTON [US]; LILLY CO ELI [US]; HOLTZMAN DAVID M [US]; DEMATT) 30 August 2001 (2001-08-30) sequences 9-10 examples 1-17	1-14,17, 18,20, 21,23, 28-32, 34-37, 39,40, 42,43, 45-50, 69-72, 74,75, 86,87, 91,92, 97-100, 104
A	DATABASE BIOSIS [Online] BIOSCIENCES INFORMATION SERVICE, PHILADELPHIA, PA, US; 2003, SEUBERT P ET AL: "Comparative efficacy of different immunotherapeutic approaches in reducing AD - like neuropathology." XP002495591 Database accession no. PREV200400194890 abstract & SOCIETY FOR NEUROSCIENCE ABSTRACT VIEWER AND ITINERARY PLANNER, vol. 2003, 2003, pages Abstract No. 133.3 URL-http://sf, 33RD ANNUAL MEETING OF THE SOCIETY OF NEUROSCIENCE; NEW ORLEANS, LA, USA; NOVEMBER 08-12, 2003 -/--	

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6725

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2008/065199

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No:
P,X	WO 2007/064972 A (ABBOTT LAB [US]; ABBOTT GMBH & CO KG [DE]; LABKVOSKY BORIS [US]; BARGH) 7 June 2007 (2007-06-07) sequences 3-10 figures 5,6 examples 1-6	1-160
P,X	WO 2007/068412 A (AC IMMUNE SA [CH]; GREFERATH RUTH [DE]; HICKMAN DAVID [CH]; MUHS ANDRE) 21 June 2007 (2007-06-21) page 103 examples 1-8,11-17 sequences 7-8,21-22	1-106

Form PCT/ISA/210 (continuation of second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2008/065199

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2006094724 A	14-09-2006	AU 2006222193 A1	14-09-2006
		CA 2599792 A1	14-09-2006
		JP 2008531635 T	14-08-2008
		KR 20080021585 A	07-03-2008
WO 03090772 A	06-11-2003	AU 2003223474 A1	10-11-2003
		CA 2483729 A1	06-11-2003
		EP 1501531 A1	02-02-2005
		JP 2005523335 T	04-08-2005
WO 0162801 A	30-08-2001	AT 279442 T	15-10-2004
		AU 4178601 A	03-09-2001
		BR 0108676 A	07-01-2003
		CA 2400559 A1	30-08-2001
		CN 1426423 A	25-06-2003
		CZ 20022851 A3	17-09-2003
		DE 1257584 T1	28-05-2003
		DE 60106394 D1	18-11-2004
		DE 60106394 T2	09-03-2006
		EP 1257584 A2	20-11-2002
		ES 2184660 T1	16-04-2003
		HK 1048640 A1	01-04-2005
		HR 20020693 A2	31-12-2004
		HU 0204074 A2	28-03-2003
		JP 2003523764 T	12-08-2003
		MX PA02008145 A	05-04-2004
		NO 20023957 A	22-10-2002
		NZ 520800 A	30-04-2004
		PL 356798 A1	12-07-2004
		PT 1257584 T	31-01-2005
		SK 12212002 A3	07-10-2003
		TR 200202799 T3	21-03-2003
		US 2004043418 A1	04-03-2004
WO 2007064972 A	07-06-2007	AU 2006320392 A1	07-06-2007
WO 2007068412 A	21-06-2007	AR 058326 A1	30-01-2008
		AU 2006326284 A1	21-06-2007
		EP 1959996 A2	27-08-2008
		US 2007166311 A1	19-07-2007

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6727

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
24 December 2008 (24.12.2008)

PCT

(10) International Publication Number
WO 2008/156622 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *C07K 16/18* (2006.01)
A61P 25/28 (2006.01)

(21) International Application Number:
PCT/US2008/007318

(22) International Filing Date: 12 June 2008 (12.06.2008)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/943,509 12 June 2007 (12.06.2007) US

(71) Applicants *(for all designated States except US)*: **AC IMMUNE S.A.** [CH/CH]; PSE Building B-EPFL, CH-1015 Lausanne (CH). **GENENTECH, INC.** [US/US]; 1 DNA Way, South San Francisco, CA 94080-4990 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: **PFEIFER, Andrea** [DE/SE]; Route de Fenil 16A, CH-1806 St-Légier (CH). **PIHLGREN, Maria** [SE/SE]; Rue du Centre 42A, CH-1025 St. Sulpice (CH). **MUHS, Andreas** [DE/SE]; Avenue des Cerisiers 39b, CH-1009 Pully (CH). **WATTS, Ryan** [US/US]; 524 Yale Drive, San Mateo, CA 94402 (US).

(74) Agents: **PEET, Richard, C.** et al.; Foley & Lardner, Suite 500, 3000 K Street, N.W., Washington, DC 20007 (US).

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

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

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

WO 2008/156622 A1

(54) Title: HUMANIZED ANTIBODIES TO AMYLOID BETA

(57) Abstract: The present application is related to chimeric and humanized antibody and to methods and compositions for the therapeutic and diagnostic use in the treatment of amyloidosis, a group of disorders and abnormalities associated with amyloid protein such as Alzheimer's disease.

HUMANIZED ANTIBODIES TO AMYLOID BETA

CROSS-REFERENCE TO RELATED PATENT APPLICATIONS

[0001] This application claims priority to United States Provisional Applications Serial No. 60/943,509 filed on June 12, 2007, which is incorporated herein by reference.

BACKGROUND OF THE INVENTION

[0002] The present invention is related to methods and compositions for diagnosis and treatment of amyloidosis, a group of disorders and abnormalities associated with amyloid protein such as Alzheimer's disease.

[0003] Amyloidosis is not a single disease entity but rather a diverse group of progressive disease processes characterized by extracellular tissue deposits of a waxy, starch-like protein called amyloid, which accumulates in one or more organs or body systems. As the amyloid deposits accumulate, they begin to interfere with the normal function of the organ or body system. There are at least 15 different types of amyloidosis. The major forms are primary amyloidosis without known antecedent, secondary amyloidosis following some other condition, and hereditary amyloidosis.

[0004] Secondary amyloidosis occurs during chronic infection or inflammatory disease, such as tuberculosis, a bacterial infection called familial Mediterranean fever, bone infections (osteomyelitis), rheumatoid arthritis, inflammation of the small intestine (granulomatous ileitis), Hodgkin's disease, and leprosy.

[0005] Amyloid deposits include amyloid P (pentagonal) component (AP), a glycoprotein related to normal serum amyloid P (SAP), and sulphated glycosaminoglycans (GAG), complex carbohydrates of connective tissue. Amyloid protein fibrils, which account for about 90% of the amyloid material, comprise one of several different types of proteins. These proteins are capable of folding into so-called "beta-pleated" sheet fibrils, a unique protein configuration which exhibits binding sites for Congo red resulting in the unique staining properties of the amyloid protein.

[0006] Many diseases of aging are based on or associated with amyloid-like proteins and are characterized, in part, by the buildup of extracellular deposits of amyloid or amyloid-like

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6729

material that contribute to the pathogenesis, as well as the progression of the disease. These diseases include, but are not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex. Other diseases which are based on or associated with amyloid-like proteins are progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration.

[0007] Although pathogenesis of these diseases may be diverse, their characteristic deposits often contain many shared molecular constituents. To a significant degree, this may be attributable to the local activation of pro-inflammatory pathways thereby leading to the concurrent deposition of activated complement components, acute phase reactants, immune modulators, and other inflammatory mediators (McGeer et al., 1994).

[0008] Alzheimer's Disease (AD) is a neurological disorder primarily thought to be caused by amyloid plaques, an accumulation of abnormal deposit of proteins in the brain. The most frequent type of amyloid found in the brain of affected individuals is composed primarily of $A\beta$ fibrils. Scientific evidence demonstrates that an increase in the production and accumulation of beta-amyloid protein in plaques leads to nerve cell death, which contributes to the development and progression of AD. Loss of nerve cells in strategic brain areas, in turn, causes reduction in the neurotransmitters and impairment of memory. The proteins principally responsible for the plaque build up include amyloid precursor protein (APP) and two presenilins (presenilin I and presenilin II). Sequential cleavage of the amyloid precursor protein (APP), which is constitutively expressed and catabolized in most cells, by the enzymes β and γ secretase leads to the release of a 39 to 43 amino acid $A\beta$ peptide. The degradation of APPs likely increases their propensity to aggregate in plaques. It is especially the $A\beta(1\text{-}42)$ fragment that has a high propensity of building aggregates due to two very hydrophobic amino acid residues at its C-terminus. The $A\beta(1\text{-}42)$ fragment is therefore believed to be mainly involved and responsible for the initiation of neuritic plaque formation in AD and to have, therefore, a high pathological potential. There is therefore a need for agents to prevent the formation of amyloid plaques and to diffuse existing plaques in AD.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6730

[0009] The symptoms of AD manifest slowly and the first symptom may only be mild forgetfulness. In this stage, individuals may forget recent events, activities, the names of familiar people or things and may not be able to solve simple math problems. As the disease progresses, symptoms are more easily noticed and become serious enough to cause people with AD or their family members to seek medical help. Mid-stage symptoms of AD include forgetting how to do simple tasks such as grooming, and problems develop with speaking, understanding, reading, or writing. Later stage AD patients may become anxious or aggressive, may wander away from home and ultimately need total care.

[0010] Presently, the only definite way to diagnose AD is to identify plaques and tangles in brain tissue in an autopsy after death of the individual. Therefore, doctors can only make a diagnosis of "possible" or "probable" AD while the person is still alive. Using current methods, physicians can diagnose AD correctly up to 90 percent of the time using several tools to diagnose "probable" AD. Physicians ask questions about the person's general health, past medical problems, and the history of any difficulties the person has carrying out daily activities. Behavioral tests of memory, problem solving, attention, counting, and language provide information on cognitive degeneration and medical tests such as tests of blood, urine, or spinal fluid, and brain scans can provide some further information.

[0011] The management of AD consists of medication-based and non-medication based treatments. Treatments aimed at changing the underlying course of the disease (delaying or reversing the progression) have so far been largely unsuccessful. Medicines that restore the deficit (defect), or malfunctioning, in the chemical messengers of the nerve cells (neurotransmitters), in particular the cholinesterase inhibitors (ChEIs) such as tacrine and rivastigmine, have been shown to improve symptoms. ChEIs impede the enzymatic degradation of neurotransmitters thereby increasing the amount of chemical messengers available to transmit the nerve signals in the brain.

[0012] For some people in the early and middle stages of the disease, the drugs tacrine (COGNEX®, Morris Plains, NJ), donepezil (ARICEPT®, Tokyo, JP), rivastigmine (EXELON®, East Hanover, NJ), or galantamine (REMINYL®, New Brunswick, NJ) may help prevent some symptoms from becoming worse for a limited time. Another drug, memantine (NAMENDA®, New York, NY), has been approved for treatment of moderate to severe AD. Medications are also available to address the psychiatric manifestations of AD. Also, some

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6731

medicines may help control behavioral symptoms of AD such as sleeplessness, agitation, wandering, anxiety, and depression. Treating these symptoms often makes patients more comfortable and makes their care easier for caregivers. Unfortunately, despite significant treatment advances showing that this class of agents is consistently better than a placebo, the disease continues to progress, and the average effect on mental functioning has only been modest. Many of the drugs used in AD medication such as, for example, ChEIs also have side effects that include gastrointestinal dysfunction, liver toxicity and weight loss.

[0013] Another disease that is based on or associated with the accumulation and deposit of amyloid-like protein is macular degeneration.

[0014] Macular degeneration is a common eye disease that causes deterioration of the macula, which is the central area of the retina (the paper-thin tissue at the back of the eye where light-sensitive cells send visual signals to the brain). Sharp, clear, 'straight ahead' vision is processed by the macula. Damage to the macula results in the development of blind spots and blurred or distorted vision. Age-related macular degeneration (AMD) is a major cause of visual impairment in the United States and for people over age 65 it is the leading cause of legal blindness among Caucasians. Approximately 1.8 million Americans age 40 and older have advanced AMD, and another 7.3 million people with intermediate AMD are at substantial risk for vision loss. The government estimates that by 2020 there will be 2.9 million people with advanced AMD. Victims of AMD are often surprised and frustrated to find out how little is known about the causes and treatment of this blinding condition.

[0015] There are two forms of macular degeneration: dry macular degeneration and wet macular degeneration. The dry form, in which the cells of the macula slowly begin to break down, is diagnosed in 85 percent of macular degeneration cases. Both eyes are usually affected by dry AMD, although one eye can lose vision while the other eye remains unaffected. Drusen, which are yellow deposits under the retina, are common early signs of dry AMD. The risk of developing advanced dry AMD or wet AMD increases as the number or size of the drusen increases. It is possible for dry AMD to advance and cause loss of vision without turning into the wet form of the disease; however, it is also possible for early-stage dry AMD to suddenly change into the wet form.

[0016] The wet form, although it only accounts for 15 percent of the cases, results in 90 percent of the blindness, and is considered advanced AMD (there is no early or intermediate

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6732

stage of wet AMD). Wet AMD is always preceded by the dry form of the disease. As the dry form worsens, some people begin to have abnormal blood vessels growing behind the macula. These vessels are very fragile and will leak fluid and blood (hence 'wet' macular degeneration), causing rapid damage to the macula.

[0017] The dry form of AMD will initially often cause slightly blurred vision. The center of vision in particular may then become blurred and this region grows larger as the disease progresses. No symptoms may be noticed if only one eye is affected. In wet AMD, straight lines may appear wavy and central vision loss can occur rapidly.

[0018] Diagnosis of macular degeneration typically involves a dilated eye exam, visual acuity test, and a viewing of the back of the eye using a procedure called fundoscopy to help diagnose AMD, and—if wet AMD is suspected—fluorescein angiography may also be performed. If dry AMD reaches the advanced stages, there is no current treatment to prevent vision loss. However, a specific high dose formula of antioxidants and zinc may delay or prevent intermediate AMD from progressing to the advanced stage. Macugen® (pegaptanib sodium injection), laser photocoagulation and photodynamic therapy can control the abnormal blood vessel growth and bleeding in the macula, which is helpful for some people who have wet AMD; however, vision that is already lost will not be restored by these techniques. If vision is already lost, low vision aids exist that can help improve the quality of life.

[0019] One of the earliest signs of age-related macular degeneration (AMD) is the accumulation of extracellular deposits known as drusen between the basal lamina of the retinal pigmented epithelium (RPE) and Bruch's membrane (BM). Recent studies conducted by Anderson et al. have confirmed that drusen contains amyloid beta. (Experimental Eye Research 78 (2004) 243-256).

[0020] Ongoing research continues with studies exploring environmental, genetic, and dietary factors that may contribute to AMD. New treatment strategies are also being explored, including retinal cell transplants, drugs that will prevent or slow down the progress of the disease, radiation therapy, gene therapies, a computer chip implanted in the retina that may help stimulate vision and agents that will prevent the growth of new blood vessels under the macula.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6733

[0021] An important factor to consider when developing new drugs is the ease of use for the target patients. Oral drug delivery, -specifically tablets, capsules and softgels-, account for 70% of all dosage forms consumed because of patient convenience. Drug developers agree that patients prefer oral delivery rather than subjecting themselves to injections or other, more invasive forms of medicinal administration. Formulations resulting in low dosing intervals (i.e. once a day or sustained release) are also preferable. The ease of administering antibiotics in oral dosage forms results in an increase of patient compliance during treatment.

[0022] What is needed are effective methods and compositions for preventing or addressing the complications associated with amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration. In particular what is needed are agents capable of counteracting the physiological manifestations of the disease such as the formation of plaques associated with aggregation of fibers of the amyloid or amyloid-like peptide.

[0023] Anti-amyloid antibodies elicited by the inoculation of $A\beta_{1-42}$ mixed with Freund complete or incomplete adjuvant were reported to reduce the amyloid burden in transgenic mice for human Alzheimer disease (Schenk et al., 1999). Intraperitoneal inoculation of tetrapalmitoylated $A\beta_{1-16}$ reconstituted in liposomes to NORBA transgenic mice elicited significant titers of anti-amyloid antibodies, which were reported to solubilize amyloid fibers and plaques *in vitro* and *in vivo*. (Nicolau et al., 2002).

[0024] A possible mechanism by which the dissolution of amyloid plaques and fibres occurred was first suggested by Bard et al., (2000), who concluded that the antibodies opsonized the plaques, which were subsequently destroyed by the macrophages of the microglia. De Mattos et al., (2001) indicated that a mAb directed against the central domain of β-amyloid was able to bind and completely sequester plasma amyloid. They argued that the presence of these mAbs in circulation shifted the equilibrium of $A\beta$ between brain and

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6734

plasma, favoring the peripheral clearing and catabolism instead of deposition within the brain.

[0025] Prolonged human therapy with rodent antibodies may result in an antiglobulin response which is detectable at about 8-12 days after administration and reaches a peak at about 20-30 days. If such an antiglobulin response is encountered, the treatment must be discontinued after not more than about 10 days and re-treatment at a latter date is usually precluded because it will lead to rapid onset of a secondary antiglobulin response. Although rodent antibodies share a considerable degree of sequence conservation with that of human antibodies, there are many sequence differences between rodents and human antibodies sufficient for the rodent antibodies to be immunogenic in humans.

[0026] This problem may be overcome by generating antibodies directly in humans or by the creation of "humanized' (a.k.a. "reshaped' antibodies). Humanized antibodies have a variable region amino acid sequence that contains the rodent-derived CDRs interspersed into human or human-like framework sequences. Since the specificity of the humanized antibody is provided by the rodent-derived CDRs, their residues are to be used essentially unchanged with only minor modifications being allowable, which do not significantly interfere with the affinity and specificity of the antibody for its target antigen. Framework residues may be derived from any primate or, particularly, from any human variable region or may be a combination thereof and the resultant designed variable region would be considered reshaped.

[0027] To maximise the likelihood that affinity will be retained in the reshaped antibody it is important to make a proper selection of the framework region. It is known that the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding. In order to maintain the affinity of the antibody for its antigen it is advantageous to select human framework sequences that are most similar to the sequences of the rodent frameworks. It then may still be necessary to replace one or more amino acids in the human framework sequence with the corresponding residue in the rodent framework to avoid losses with the affinity. This replacement may be aided by computer modelling.

[0028] The present invention provides novel methods and compositions comprising highly specific and highly effective antibodies, particularly chimeric antibodies including fragments thereof, more particularly partially or fully humanized antibodies including fragments

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6735

thereof, having the ability to specifically recognize and bind to specific epitopes from a range of β-amyloid antigens, which may be presented to the antibody in a monomeric, dimeric, trimeric, etc, a polymeric form, in form of an aggregate, fibers, filaments or in the condensed form of a plaque. The antibodies enabled by the teaching of the present invention are particularly useful for the treatment of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, to name just a few.

SUMMARY OF THE INVENTION

[0029] In one embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, and more particularly to at least three distinct binding sites on the β-amyloid protein wherein said one, said at least two and said at least three binding sites each comprise at least one or two consecutive amino acid residues predominantly involved in the binding of the antibody.

[0030] In particular, the chimeric antibody or a fragment thereof, or the humanized antibody or a fragment thereof according to the invention binds to at least two, particularly to at least three distinct binding sites on the β-amyloid protein wherein at least two of the three distinct binding sites comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody and at least one the three distinct binding sites comprise at least one amino acid residue.

[0031] The at least two distinct binding sites comprising at least two consecutive amino acid residues predominantly involved in the binding of the antibody are located in close proximity to each other on the antigen, separated and/or flanked by at least one amino acid

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6736

residue not involved in antibody binding or to a significantly smaller extent as compared to said at least two consecutive amino acid residues, thus forming a conformational discontinuous epitope.

[0032] The at least three distinct binding sites comprising at least two consecutive amino acid residues and at least one amino acid residue, respectively, which are predominantly involved in the binding of the antibody are located in close proximity to each other on the epitope, separated and/or flanked by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues, which are predominantly involved in the binding of the antibody, thus forming a conformational discontinuous epitope.

[0033] In particular, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said at least one or said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- embedded within the following core sequence (SEQ ID NO: 9):

Xaa_3 - Phe – Phe – Xaa_4 – Xaa_5 – Xaa_6, wherein

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_3 Xaa_4, Xaa_5 and Xaa_6 are not involved in antibody binding or to a significantly smaller extent as compared to the –Phe-Phe- binding site.

[0034] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6737

Xaa_5 is Glu or Asp, but particularly Glu;

Xaa_6 is Glu or Asp, but particularly Asp.

[0035] In particular, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a first binding site are –Phe-Phe- and the at least one amino acid residue is –His- embedded within the following core sequence:

$-Xaa_1-His-Xaa_3-Xaa_4-Xaa_5-Xaa_6-Phe-Phe-Xaa_7-Xaa_8-Xaa_9-$,

wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg

Xaa_3 is an amino acid residue selected from the group consisting of Asn and Gln

Xaa_4 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg

Xaa_5 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_7 is an amino acid residue selected from the group consisting of Ala, Val, Leu and Ile

Xaa_8 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_9 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_1, Xaa_3, Xaa_6, Xaa_7, Xaa_8 and Xaa_9, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His- and the –Phe-Phe- binding site, respectively.

[0036] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_3 is Gln or Asn, but particularly Gln;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6738

Xaa_4 is Lys

Xaa_5 is Leu

Xaa_6 is Val or Leu, but particularly Val;

Xaa_7 is Ala or Val, but particularly Ala;

Xaa_8 is Glu or Asp, but particularly Glu; and

Xaa_9 is Asp or Glu, but particularly Asp.

[0037] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein, wherein said at least one or said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acid residues representing a second binding site are –Lys-Leu- embedded within the following core sequence (SEQ ID NO: 10):

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile; and wherein said amino acid residues Xaa_2, Xaa_3, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –Lys-Leu- binding site.

[0038] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids, which are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6739

the amino acid residues predominantly involved in the binding of the antibody, are -His- and –Lys-Leu-, respectively, embedded within the following core sequence:

His – Xaa_2 - Lys - Leu – Xaa_3– Xaa_4– Xaa_5–Xaa_6- – Xaa_7 — Xaa_8 – wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_5 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile

Xaa_6 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_7 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_8 is an amino acid residue selected from the group consisting of Glu and Asp

and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_6, Xaa_7, Xaa_8, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His- and the –Lys-Leu- binding site, respectively.

[0039] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Phe

Xaa_5 is Phe

Xaa_6 is Ala or Val, but particularly Ala;

Xaa_7 is Glu or Asp, but particularly Glu; and

Xaa_8 is Asp or Glu, but particularly Asp.

[0040] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acids are

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6740

separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent than said consecutive amino acid residues, which are –Phe-Phe- and –Lys-Leu-, respectively, representing a first and second binding site embedded within the following core sequence:

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 - Phe – Phe - $Xaa_4 – Xaa_5 – Xaa_6$, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln Lys, and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5 and Xaa_6 are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the the –Lys-Leu- and –Phe-Phe- binding site, respectively.

[0041] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein said distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, wherein the at least one and the at least two consecutive amino acids are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent as compared to the amino acid residues, which are predominantly involved in the binding of the antibody, and wherein said amino acid residues are –His- and –Phe-Phe- and –Lys-Leu-, respectively, embedded within the following core sequence:

His – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe - $Xaa_4 \_ Xaa_5 \_ Xaa_6$, wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6741

Xaa_3 is an amino acid residue selected from the group consisting of Ala, Val, Leu, norleucine, Met, Phe, and Ile;

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu, Ser and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the –His-, the –Lys-Leu- and the –Phe-Phe- binding site, respectively.

[0042] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Xaa_5 is Glu or Asp, but particularly Glu; and

Xaa_6 is Asp or Glu, but particularly Asp.

[0043] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, wherein the at least two consecutive amino acids are separated by at least one amino acid residue not involved in antibody binding or to a significantly smaller extent than said consecutive amino acid residues, which are –Phe-Phe- and –Lys-Leu-, respectively, representing a first and second binding site embedded within the following core sequence:

$Xaa_1 – Xaa_2$ - Lys - Leu – Xaa_3 - Phe – Phe - $Xaa_4 – Xaa_5 – Xaa_6$, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, Lys and Arg;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln;

Xaa_3 is an amino acid residue selected from the group consisting of Val, Ala, Leu, Met, Phe, norleucine and Ile

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6742

Xaa_4 is an amino acid residue selected from the group consisting of Ala, Val, Leu and Ile;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp,

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller to significantly smaller extent as compared to the —Lys-Leu- and the –Phe- Phe binding site, respectively.

[0044] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein

Xaa_1 is His or Arg, but particularly His;

Xaa_2 is Gln or Asn, but particularly Gln;

Xaa_3 is Val or Leu, but particularly Val;

Xaa_4 is Ala or Val, but particularly Ala;

Xaa_5 is Glu or Asp, but particularly Glu; and

Xaa_6 is Asp or Glu, but particularly Asp.

[0045] In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues predominantly involved in the binding of the antibody, which are - Phe - Phe – Ala – Glu -, particularly - Phe - Phe – Ala –,but especially - Phe - Phe – and - Lys - Leu –, respectively, and wherein said at least two distinct binding sites exhibit amino acid sequence –Val - Phe - Phe - Ala - Glu – Asp - shown in SEQ ID NO: 7 and amino acid sequence His - Gln - Lys - Leu – Val - shown in SEQ ID NO: 8, respectively.

[0046] In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, which recognizes and binds to at least one distinct binding site, particularly to a least two distinct binding sites, more particularly to at least three distinct binding sites on the β-amyloid protein wherein the said at least one or said at least two distinct binding sites comprise at least one and at least two consecutive amino acid residues, respectively, predominantly involved in the binding of the antibody, which are - Phe - Phe – and - Lys - Leu –, and –His-, respectively, wherein said distinct

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6743

binding sites are embedded in the amino acid sequence –Val - Phe - Phe - Ala – Glu-, and amino acid sequence -His - Gln - Lys - Leu – Val -, respectively.

[0047] In another embodiment of the invention, the chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof comprises an antigen recognition and binding site which recognizes and binds to at least two distinct binding sites on the β-amyloid protein wherein said at least two distinct binding sites each comprise at least two consecutive amino acid residues within the amino acid sequence given in SEQ ID NOs: 7 and 8, respectively, wherein said consecutive amino acid residues, particularly –Phe- Phe- and –Lys-Leu-, are predominantly involved in the binding of the β-amyloid protein.

[0048] In a specific embodiment of the invention, the recognition and binding sites as defined herein before are forming a conformational discontinuous epitope localized in a region of the β-amyloid protein between amino acid residue 12 to 24, particularly between residues 14 to 23, more particularly between amino acid residues 14 and 20, wherein the at least two distinct recognition and binding sites each comprising at least 2 amino acid residues, are located at position 16 and 17 and at position 19 and 20, respectively, and wherein the at least one distinct recognition and binding site comprising at least 1 amino acid residue is located at position 14, which residues are predominantly involved in the binding of the β-amyloid protein and wherein said distinct recognition and binding sites are at least on one side flanked by amino acid residues, particularly residues 21 and 22, and separated by one amino acid residue located at position 15 and 18, which amino acid residues are not directly involved in the binding of the antigen or, at least, to a substantially smaller extent.

[0049] In still another embodiment of the invention the said at least three distinct recognition and binding sites are flanked on both sides by amino acid residues, particularly residues 12 and 13, and residues 21 and 22 and are separated by one amino acid residue located at position 15 and 18, which amino acid residues are not directly involved in the binding of the antigen or, at least, to a substantially smaller extent.

[0050] In a specific embodiment, said consecutive amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region:

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6744

Val–	His–	His–	Gln–	Lys–	Leu–	Val–	Phe–	Phe–	Ala–	Glu–	Asp
12	13	14	15	16	17	18	19	20	21	22	23

[0051] In another specific embodiment, said amino acid residues, particularly –Lys-Leu- at position 16 and 17 and –Phe- Phe- at position 19 and 20, and –His- at position 14, which are predominantly involved in the binding of the β-amyloid protein, are embedded into the following core region:

Val–	His–	His–	Gln–	Lys–	Leu–	Val–	Phe–	Phe–	Ala–	Glu–	Asp–	Val–	Gly–
12	13	14	15	16	17	18	19	20	21	22	23	24	25

[0052] In another embodiment of the invention, a humanized antibody or a fragment thereof is provided which comprises in the light chain and heavy chain variable region, respectively, at least one CDR of non-human origin, particularly two CDRs of non-human origin, more particularly three CDR of non-human origin, embedded in one or more human- or primate-derived framework regions and, optionally, a constant region derived from a human or primate source antibody, which humanized antibody or fragment thereof is capable of specifically recognizing and binding β-amyloid protein, particularly a β-amyloid monomeric peptide, more particularly a β-amyloid polymeric peptide, even more particularly β-amyloid fibers, fibrils or filaments in isolation or as part of a β-amyloid plaque, at an epitope comprising the following amino acid sequence (SEQ ID NO: 11):

Xaa_1 – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe- Xaa_4 - Xaa_5 - Xaa_6, wherein

Xaa_1 is an amino acid residue selected from the group consisting of His, Asn, Gln, but particularly His;

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln; and

Xaa_3 is an amino acid residue selected from the group consisting of Val, Leu, and Ile, but particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala and Val, but particularly Ala;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6745

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu;

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Asp.

[0053] In still another embodiment of the invention, a humanized antibody or a fragment thereof is provided which comprises in the light chain and heavy chain variable region, respectively, at least one CDR of non-human origin, particularly two CDRs of non-human origin, more particularly three CDR of non-human origin, embedded in one or more human- or primate-derived framework regions and, optionally, a constant region derived from a human or primate source antibody, which humanized antibody or fragment thereof is capable of specifically recognizing and binding β-amyloid protein, particularly a β-amyloid monomeric peptide, more particularly a β-amyloid polymeric peptide, even more particularly β-amyloid fibers, fibrils or filaments in isolation or as part of a β-amyloid plaque, at an epitope comprising the following amino acid sequence:

His – Xaa_2 - Lys - Leu – Xaa_3 - Phe – Phe- Xaa_4 - Xaa_5 - Xaa_6, wherein

Xaa_2 is an amino acid residue selected from the group consisting of Asn and Gln, but particularly Gln; and

Xaa_3 is an amino acid residue selected from the group consisting of Val, Leu, and Ile, but particularly Val;

Xaa_4 is an amino acid residue selected from the group consisting of Ala and Val, but particularly Ala;

Xaa_5 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu;

Xaa_6 is an amino acid residue selected from the group consisting of Glu and Asp, but particularly Glu; and wherein said amino acid residues Xaa_2, Xaa_3, Xaa_4, Xaa_5, Xaa_6, are not involved in antibody binding or to a smaller extent as compared to the –His- and the –Lys-Leu- and the -Phe-Phe- binding site.

[0054] In a specific embodiment of the invention, the CDR of non-human origin is obtained from a donor antibody, but particularly from a murine donor antibody, raised against an antigen fragment which does not contain said distinct binding site. This shift in the epitopic region may have at least partially been caused by the use of a supramolecular antigenic

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6746

construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide, particularly of β-amyloid peptide $A\beta_{1-16}$, modified with a hydrophilic moiety such as, for example, polyethylene glycol (PEG), wherein said hydrophilic moiety is covalently bound to each of the termini of the antigenic peptide through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophilic moiety to the peptide fragment, as described herein below in the immunization process. When a PEG is used as the hydrophilic moiety, the free PEG termini are covalently bound to phosphatidylethanolamine or any other compound suitable to function as the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome as described herein.

[0055] In particular, the CDR of non-human origin is obtained from a murine donor antibody which exhibits the characteristic properties of ACI-01-Ab7C2 (also named "mC2" throughout the application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty under accession no DSM ACC2750).

[0056] In one embodiment of the invention, the CDR of non-human origin is obtained from murine donor antibody ACI-01-Ab7C2 (also named "mC2" throughout the application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty under accession no DSM ACC2750).

[0057] Also the use of lipid A as part of the immunization protocol may have contributed to a shift in the epitopic region.

[0058] In a specific embodiment, the invention relates to a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6747

[0059] In another embodiment, the invention relates to a humanized antibody or a fragment thereof, wherein said humanized antibody comprises integrated into human- or primate-derived heavy chain framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0060] In still another embodiment, the invention relates to a humanized antibody or a fragment thereof, wherein said humanized antibody comprises integrated into human- or primate-derived light chain framework regions a peptide with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

[0061] In particular, the invention relates to a Light Chain Variable Region (LCVR) comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence of SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

[0062] In another specific embodiment, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived framework regions at least one peptide with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0063] The invention further relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least two peptides, which peptides are different and exhibit an amino acid sequence selected from the group of sequences consisting of SEQ ID NO:1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR) wherein the same CDR cannot be present twice in the antibody. In particular, if the at least two CDRs present are both CDRs of the Light Chain Variable Region (LCVR), at least on of said CDRs must be CDR1 represented by SEQ ID NO: 4.

[0064] Also comprised by the invention is a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6748

SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

[0065] In particular, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0066] In a further embodiment, the invention relates to a humanized antibody or a fragment thereof, comprising integrated into human- or primate-derived light chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

[0067] In particular, the invention relates to a Light Chain Variable Region (LCVR), which has integrated into human- or primate-derived light chain framework regions at least two peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), wherein the same CDR cannot be present twice in the antibody and, in particular, at least on of said CDRs must be CDR1 represented by SEQ ID NO: 4.

[0068] The invention also relates to a humanized antibody or a fragment thereof, comprising integrated into human- or primate-derived heavy chain framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated above.

[0069] In particular, the invention relates to a Heavy Chain Variable Region (HCVR) comprising integrated into human- or primate-derived heavy chain framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR), particularly in the order indicated above.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6749

[0070] Also comprised by the invention is a humanized antibody or a fragment thereof comprising integrated into human- or primate-derived light chain framework regions peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated above.

[0071] In particular, the invention relates to a Light Chain Variable Region (LCVR) comprising integrated into human- or primate-derived light chain framework regions peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), particularly in the order indicated above.

[0072] The invention also relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least three peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

[0073] In another embodiment the invention relates to a humanized antibody or a fragment thereof, which antibody comprises integrated into human- or primate-derived framework regions at least four peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1 , SEQ ID NO: 2 representing CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

[0074] In still anther embodiment, the invention relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions at least five peptides with an amino acid sequence selected from the group of sequences consisting of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6750

CDR2 and SEQ ID NO:3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR), but particularly a humanized antibody or a fragment thereof wherein the same CDR cannot be present twice in the antibody.

[0075] In still anther embodiment, the invention relates to a humanized antibody or a fragment thereof, which comprises integrated into human- or primate-derived framework regions peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1, SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR) and SEQ ID NO: 4 representing CDR1, SEQ ID NO: 5 representing CDR2 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

[0076] In a specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR), or a fragment thereof, wherein said humanized antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least a peptide with an amino acid sequence of SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

[0077] In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, wherein said humanized antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least a peptide with an amino acid sequence of SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0078] In another specific embodiment, the invention relates to a humanized antibody, Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 2 representing CDR2 of the Heavy Chain Variable Region (HCVR).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6751

[0079] In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 1 representing CDR1 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0080] In another specific embodiment, the invention relates to a humanized antibody, a Heavy Chain Variable Region (HCVR) or a fragment thereof, which antibody, Heavy Chain Variable Region (HCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 2 representing CDR2 and SEQ ID NO: 3 representing CDR3 of the Heavy Chain Variable Region (HCVR).

[0081] In another specific embodiment, the invention relates to a humanized antibody or a fragment thereof comprising variable regions with human- or primate-derived framework regions and at least one CDR with an amino acid sequence selected from the group consisting of SEQ ID NO:1 representing CDR1 of the Heavy Chain Variable Region (HCVR), SEQ ID NO:2 representing CDR2 of the Heavy Chain Variable Region (HCVR), and SEQ ID NO: 4 representing CDR1 of the Light Chain Variable Region (LCVR).

[0082] In another specific embodiment, the invention relates to a humanized antibody, a Light Chain Variable Region (LCVR) or a fragment thereof, which antibody, Light Chain Variable Region (LCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 5 representing CDR2 of the Light Chain Variable Region (LCVR).

[0083] In another specific embodiment, the invention relates to a humanized antibody, a Light Chain Variable Region (LCVR) or a fragment thereof, which antibody, Light Chain Variable Region (LCVR) or fragment thereof comprises integrated into human- or primate-derived heavy chain framework regions at least two peptides with an amino acid sequence of SEQ ID NO: 4 representing CDR1 and SEQ ID NO: 6 representing CDR3 of the Light Chain Variable Region (LCVR).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6752

[0084] Further comprised by the invention is a humanized antibody or a fragment thereof, wherein both the Heavy Chain Variable Region (HCVR) and the Light Chain Variable Region (LCVR) of the mouse C2 antibody each contributes at least one of its CDR regions to the at least two CDR regions of the humanized antibody. The resulting humanized antibody or a fragment thereof thus may comprise

-at least an amino acid sequence of SEQ ID NO: 1 representing CDR1 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 4 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 1 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR2 (LCVR);

-at least an amino acid sequence of SEQ ID NO:2 representing CDR2 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR3 (LCVR);

-at least an amino acid sequence of SEQ ID NO:1 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR1 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 5 representing CDR2 (LCVR);

-at least an amino acid sequence of SEQ ID NO: 3 representing CDR3 (HCVR) in combination with an amino acid sequence of SEQ ID NO: 6 representing CDR3 (LCVR).

[0085] In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof as described herein before, which antibody comprises a light chain and/or a heavy chain constant region of human or primate origin.

[0086] In a further embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein at least one, particularly at least one but not more than 5, more particularly at least one but not more than 4, even more particularly at least one but not more than 3, but especially at least one but not more than 2,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6753

of the amino acids representative of the light chain and/or heavy chain CDR regions as given in SEQ ID NOs: 1 – 6 is changed through a conservative substitution such that the antibody maintains its full functionality.

[0087] In particular, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein in CDR2 of the light chain variable region (LCVR) as given in SEQ ID NO: 5, the Lys at Kabat position 50 is replaced by an amino acid residue selected from the group consisting of Arg, Gln and Glu, particularly by Arg.

[0088] In particular, the invention relates to a light chain variable region (LCVR) wherein in CDR2 as given in SEQ ID NO: 5, the Lys at Kabat position 50 is replaced by an amino acid residue selected from the group consisting of Arg, Gln and Glu, particularly by Arg.

[0089] In another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof, wherein in CDR2 of the light chain variable region (LCVR) as given in SEQ ID NO: 5, the Ser at Kabat position 53 is replaced by an amino acid residue selected from the group consisting of Asn or Thr, but particularly by Asn.

[0090] In particular, the invention relates to a light chain variable region (LCVR) wherein in CDR2 as given in SEQ ID NO: 5, the Ser at Kabat position 53 is replaced by an amino acid residue selected from the group consisting of Asn or Thr, but particularly by Asn.

[0091] In one embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein the Heavy Chain Variable Region (HCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 15 and 16, respectively.

[0092] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided, wherein the Light Chain Variable Region (LCVR) has an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the sequence given in SEQ ID NO: 12 and 13, respectively.

[0093] In still another embodiment of the invention, a humanized antibody or a fragment thereof is provided, wherein at least two, but especially three, of the CDR regions of the Heavy Chain Variable Region (HCVR) have an amino acid sequence that is 90%, particularly

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6754

95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 1 – 3.

[0094] In a further embodiment of the invention, a humanized antibody or a fragment thereof is provided, wherein at least two, but especially three, of the CDR regions of the Light Chain Variable Region (LCVR) have an amino acid sequence that is 90%, particularly 95%, more particularly 98% identical to the corresponding CDR region as given in SEQ ID NO: 4 – 6.

[0095] In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before wherein the Heavy Chain Variable Region (HCVR) has an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence given in SEQ ID NO: 15 and 16, respectively.

[0096] In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before wherein the Light Chain Variable Region (LCVR) has an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the sequence given in SEQ ID NO: 12 and 13, respectively.

[0097] In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before, wherein at least one, particularly at least two, but especially three, of the CDR regions of the Heavy Chain Variable Region (HCVR) have an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the corresponding CDR region as given in SEQ ID NO: 1 – 3.

[0098] In still another embodiment, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention as described herein before, wherein at least one, particularly at least two, but especially three, of the CDR regions of the Light Chain Variable Region (LCVR) have an amino acid sequence that is 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the corresponding CDR region as given in SEQ ID NO: 4-6.

[0099] In still another embodiment, the invention relates to a humanized antibody according to the present invention and as described herein before, wherein at least one of the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6755

amino acids representative of the acceptor framework sequences obtained from human germline V_H and V_K sequences, respectively is changed through a substitution to an amino acid from the corresponding region of murine antibody ACI-01-Ab7C2 or a substitution conservative thereto.

[0100] In particular, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu.

[0101] The invention further relates to a humanized antibody, wherein the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser..

[0102] In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser.

[0103] The invention further relates to a humanized antibody, wherein the Gln in Kabat position 45 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Lys, Arg, Gln, and Asn, particularly by Lys and Arg, but especially by Lys.

[0104] The invention further relates to a humanized antibody, wherein the Leu in Kabat position 50 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Lys, Arg, Gln, and Asn, particularly by Lys and Arg, but especially by Lys.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6756

[0105] The invention further relates to a humanized antibody, wherein the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe,

[0106] In still another embodiment, the invention relates to a humanized antibody, wherein the Asn in Kabat position 53 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 may be replaced by an amino acid selected from the group consisting of Gln, His, Lys and Arg, but especially by His and Gln.

[0107] In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser, and the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by an amino acid selected from the group consisting of Phe, Leu, Val, Ile, and Ala, particularly by Leu and Phe, but especially by Phe.

[0108] In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Leu, norleucine, Ile, Val, Met, Ala, and Phe, particularly Leu and Ile, but especially Leu.

[0109] In still another embodiment, the invention relates to a humanized antibody, wherein the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6757

shown in SEQ ID NO: 15 is replaced by an amino acid selected from the group consisting of Ser and Thr, but especially by Ser.

[0110] In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Ser.

[0111] In still another embodiment, the invention relates to a humanized antibody, wherein the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by Phe.

[0112] In still another embodiment, the invention relates to a humanized antibody, wherein the Trp in Kabat position 47 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Leu and Ile, but especially Leu and the Arg in Kabat position 94 in the acceptor framework sequence obtained from human germline V_H sequences of KABAT subgroup V_HIII of the Heavy Chain Variable Region as shown in SEQ ID NO: 15 is replaced by Ser and the Tyr in Kabat position 87 in the acceptor framework sequence obtained from human germline V_K sequences of KABAT subgroup V_KII of the Light Chain Variable Region as shown in SEQ ID NO: 12 is replaced by Phe.

[0113] In a specific embodiment, the invention relates to the light chain variable region of SEQ ID NO: 12.

[0114] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the light chain variable region of SEQ ID NO: 12.

[0115] In a specific embodiment, the invention relates to the light chain variable region including signal sequences as shown in SEQ ID NO: 13.

[0116] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete light chain variable region including signal sequences as shown in SEQ ID NO: 13.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6758

[0117] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the light chain variable region of SEQ ID NO: 12 and the light chain constant region of SEQ ID NO: 14.

[0118] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete light chain variable region of SEQ ID NO: 13 and the light chain constant region of SEQ ID NO: 14.

[0119] In a specific embodiment, the invention relates to the heavy chain variable region of SEQ ID NO: 15.

[0120] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 15.

[0121] In a specific embodiment, the invention relates to the heavy chain variable region including signal sequences as shown in SEQ ID NO: 16.

[0122] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the complete heavy chain variable region including signal sequences as shown in SEQ ID NO: 16.

[0123] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 15 and the heavy chain constant region of SEQ ID NO: 17.

[0124] In another specific embodiment of the invention, a humanized antibody is provided, which comprises the heavy chain variable region of SEQ ID NO: 16 and the heavy chain constant region of SEQ ID NO: 17.

[0125] In one embodiment the humanized antibody according to the invention and as described herein, upon co-incubation with an Aβ monomeric peptide having at least 30, particularly at least 35, more particularly at least 38, even more particularly at least 40 amino acid residues and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said Aβ monomeric units, but especially with an Aβ_{1-42} monomeric and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said Aβ_{1-42} monomeric units, particularly at a molar concentration ratio of antibody to Aβ1-42 of up to 1:1000, but especially at a molar concentration ratio of between 1:10 and 1:100, inhibits the aggregation of the Aβ monomers to high molecular polymeric fibrils.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6759

[0126] In particular, the co-incubation of the antibody according to the invention with amyloid monomeric and/or polymeric soluble amyloid peptides is carried out for 24 hours to 60 hours, particularly for 30 hours to 50 hours, more particularly for 48 hours, but especially 24 hours, at a temperature of between 28°C and 40°C, particularly of between 32°C and 38°C, more particularly at 37°C.

[0127] In a specific embodiment of the invention, co-incubation with amyloid monomeric and/or polymeric soluble amyloid peptides is accomplished for 24 hours at a temperature of 37°C.

[0128] In particular, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof binds to $A\beta_{1-42}$ monomeric peptide and/or $A\beta$ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units and, upon co-incubation with $A\beta_{1-42}$ monomeric peptide and/or $A\beta$ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units inhibits the aggregation of the $A\beta$ monomers and/or polymers to high molecular polymeric fibrils.

[0129] In one embodiment, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the $A\beta$ monomers and/or $A\beta$ soluble polymers comprising a plurality of said $A\beta$ monomeric units to high molecular polymeric fibrils by at least 50%, particularly by at least 60%, particularly by at least 65%, more particularly by at least 75%, even more particularly by at least 80%, but especially by at least 85%-90%, or more as compared to the respective amyloid peptide monomers incubated in buffer (control), at a molar concentration ratio of antibody to $A\beta$1-42 of up to 1:1000, particularly at a molar concentration ratio of between 1:10 and 1:100, but especially at a molar concentration ratio of 1:10.

[0130] In a specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the $A\beta$ monomers and/or $A\beta$ soluble polymers comprising a plurality of said $A\beta$ monomeric units to high molecular polymeric fibrils by at least 30% at a molar concentration ratio of of antibody to $A\beta$1-42 of 1:100.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6760

[0131] In another specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof inhibits the aggregation of the Aβ monomers and/or Aβ soluble polymers comprising a plurality of said Aβ monomeric units to high molecular polymeric fibrils by at least 80% at a molar concentration ratio of of antibody to Aβ1-42 of 1:10.

[0132] Binding of the antibodies according to the invention and as described herein to amyloidogenic monomeric and/or polymeric peptides but, particularly, to the amyloid form (1-42) leads to inhibition of the aggregation of monomeric and/or polymeric amyloidogenic peptides to high molecular fibrils or filaments. Through the inhibition of the aggregation of amyloidogenic monomeric and/or polymeric peptides the antibodies according to the present invention are capable of preventing or slowing down the formation of amyloid plaques, particularly the amyloid form (1-42), which is know to become insoluble by change of secondary conformation and to be the major part of amyloid plaques in brains of diseased animals or humans.

[0133] The aggregation inhibition potential of the antibody according to the invention may be determined by any suitable method known in the art, particularly by density-gradient ultracentrifugation followed by a SDS-PAGE sedimentation analysis on a preformed gradient and/or by a thioflavin T (Th-T) fluorescent assay.

[0134] In one embodiment, the invention relates to an antibody, particularly a humanized antibody as described herein including any functionally equivalent antibody or functional parts thereof, which antibody, upon co-incubation, particularly at a molar concentration ratio of between 1:10 and 1:1000, more particularly at a ratio of 1:100 with preformed high molecular polymeric amyloid fibrils or filaments formed by the aggregation of Aβ monomeric peptides having at least 30, particularly at least 35, more particularly at least 38, even more particularly at least 40 amino acid residues and, but especially $A\beta_{1-42}$ monomeric peptides, is capable of disaggregating the preformed polymeric fibrils or filaments by at least 20%, particularly by at least 30%, more particularly by at least 35%%, even more particularly by at least 40%, but especially by at least 50% or more.

[0135] In a specific embodiment of the invention, the aggregation inhibition and the disaggregation potential of the antibody, respectively, is determined by density-gradient

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6761

ultracentrifugation followed by a SDS-PAGE sedimentation analysis on a preformed gradient.

[0136] In another specific embodiment of the invention, the aggregation inhibition and the disaggregation potential of the antibody, respectively, is determined by thioflavin T (Th-T) fluorescent assay.

[0137] In another specific embodiment, the antibody according to the invention is co-incubated with amyloid preformed high molecular polymeric amyloid fibrils or filaments for 12 hours to 36 hours, particularly for 18 hours to 30 hours, more particularly for 24 hours at a temperature of between 28°C and 40°C, particularly of between 32°C and 38°C, more particularly at 37°C.

[0138] In particular, the co-incubation with preformed high molecular polymeric amyloid fibrils or filaments is done for 24 hours at a temperature of 37°C.

[0139] In a specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof is capable of disaggregating the preformed polymeric fibrils or filaments by at least 24% at a molar concentration ratio of antibody to Aβ1-42 of 1:100.

[0140] In another specific embodiment of the invention, the antibody, particularly the humanized antibody according to the invention including any functionally equivalent antibody or functional parts thereof is capable of disaggregating the preformed polymeric fibrils or filaments by at least 32% at a molar concentration ratio of antibody to Aβ1-42 of 1:10.

[0141] Through the disaggregation of amyloidogenic polymeric fibrils or filaments the antibodies according to the present invention are capable of preventing or slowing down the formation of amyloid plaques which leads to an alleviation of the symptoms associated with the disease and a delay or reversal of its progression.

[0142] Accordingly, it is a further embodiment of the invention to provide an antibody, particularly a humanized antibody, including any functionally equivalent antibody or functional parts thereof as described herein, which antibody is capable of decreasing the total amount of Aβ in the brain of an animal, particularly a mammal, but especially a human suffering from a disease or condition leading to increased concentration of Aβ in the brain.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6762

[0143] In another embodiment, the invention relates to a humanized antibody according to the invention and as described herein before, which antibody is bi-effective in that it exhibits both an aggregation inhibition property as well as a disaggregation property, particularly paired with a high degree of conformational sensitivity.

[0144] In particular, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, which antibody, upon co-incubation with amyloid monomeric and/or polymeric soluble amyloid peptides, particularly with β-amyloid monomeric peptides such as, for example, Aβ monomeric peptides 1-39; 1-40, 1-41, or 1-42, and/or a polymeric soluble β-amyloid peptide comprising a plurality of said Aβ monomeric units, but especially with an $A\beta_{1-42}$ monomeric and/or an Aβ polymeric soluble amyloid peptide comprising a plurality of said $A\beta_{1-42}$ monomeric units, inhibits the aggregation of the Aβ monomers into high molecular polymeric fibrils or filaments and, in addition, upon co-incubation with preformed high molecular polymeric amyloid fibrils or filaments formed by the aggregation of amyloid monomeric peptides, particularly β-amyloid monomeric peptides such as, for example, Aβ monomeric peptides 1-39; 1-40, 1-41, or 1-42, but especially $A\beta_{1-42}$ monomeric peptides, is capable of disaggregating the preformed polymeric fibrils or filaments.

[0145] In another aspect, the invention relates to a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, which antibody is capable of inducing a transition of the β-sheet conformation towards an α-helix and/or a random coil conformation, but particularly a random coil conformation, even more particularly a random coil conformation at a given location in the molecule, especially in the environment of Tyr 10 and Val12 of the Aβ protein, which leads to an increase of the random coil conformation at the expense of the β-sheet conformation and an improved solubilization of the preformed high molecular polymeric amyloid fibrils or filaments. In particular the decrease of the β-sheet conformation amounts to at least 30%, particularly to at least 35%, and more particularly to at least 40% and more as compared to the respective preformed amyloid polymeric fibrils or filaments incubated in buffer (control).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6763

[0146] The antibody's potential in inducing a transition in the secondary structure is determined by solid state 13C NMR spectroscopy but, in particular, by measuring the integral intensities of the conformations of Tyr 10 and Val 12 Cβ in the Aβ_{1-42} peptide.

[0147] In a further embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof, wherein said antibody or fragment binds to an Aβ monomer with a binding affinity of at least about 1×10^{-7} to at least about 1×10^{-12}, particularly of at least about 1×10^{-8} to at least about 1×10^{-11}, more particularly of at least about 1×10^{-9} to at least about 1×10^{-10}, even more particularly of at least about 1×10^{-8} to at least about 2×10^{-8},, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

[0148] In another embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof, wherein said antibody or fragment binds to an Aβ fiber, fibril or filament with a binding affinity of at least about 1×10^{-7} to at least about 1×10^{-12}, particularly of at least about 1×10^{-8} to at least about 1×10^{-11}, more particularly of at least about 1×10^{-9} to at least about 1×10^{-10}, even more particularly of at least about 2×10^{-9} to at least about 5×10^{-9}, but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

[0149] In another embodiment, the antibody according to the invention and as described herein before or a fragment thereof, exhibits an binding affinity to an Aβ fiber, fibril or filament which is at least 10 times, particularly at least 15 times, more particularly at least 20 times, but especially at least 25 times higher than the binding affinity to an Aβ monomer.

[0150] In still another embodiment, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided as described herein before, which antibody substantially binds to aggregated Aβ, including Aβ plaques, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6764

[0151] In another aspect of the invention, the chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided as described herein before, which antibody substantially binds to soluble polymeric amyloid, particularly amyloid β (Aβ), including Aβ monomers, in the mammalian, particularly the human brain but, preferably, does not show any significant cross-reactivity with amyloid precursor protein (APP).

[0152] Further provided is a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, which antibody significantly reduces Aβ plaque burden in the mammalian, particularly the human brain. This can be achieved by either binding of the antibody to the plaque or by shifting the equilibrium between amyloid, particularly amyloid β (Aβ), in its insoluble and aggregated state towards its soluble form by disaggregating fibers to soluble poly- and monomeric forms by inducing a shift in conformation and binding and stabilizing the disaggregated and solubilized amyloid forms, particularly amyloid β (Aβ) forms, in the tissue and/or body fluids of a subject, including a mammal and a human, particularly the brain of the subject. Through the activity of the antibody according to the invention the peripheral clearing and catabolism is thus favored rather than deposition within the tissue and/or body fluids of the subject, particularly the brain. The beneficial effect of the antibody according to the invention can thus be obtained without binding of the antibody to the plaque.

[0153] Through this stabilizing activity, the antibody according to the invention is able to neutralize the toxic effects of the polymeric and less aggregated soluble amyloid protein, particularly amyloid β (Aβ) protein, in the tissue and/or body fluids of a subject, particularly of a mammal, and even more particularly of a human. In a specific embodiment of the invention the antibody according to the invention may thus achieve its beneficial effects without necessarily binding aggregated amyloid beta in the brain of the subject.

[0154] In a further aspect of the invention a humanized antibody or a fragment thereof according to the present invention and as described herein before, is provided comprising at least one light chain or a fragment thereof or at least one heavy chain or a fragment thereof incorporating at least one, particularly two and more particularly three CDR regions obtained form a mouse donor antibody, particularly from mouse antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6765

GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Braunschweig, under accession no DSM ACC2750, wherein said antibody or fragment thereof has an affinity to the Aβ antigen which is at least 5 times, particularly at least 8 times, more particularly at least 10 times, but especially at least 15 times higher than that of the mouse donor antibody.

[0155] The antibody of this invention can be, in one embodiment, a whole antibody (e.g., with two full length light chains and two full length heavy chains) of any isotype and subtype (*e.g.*, IgM, IgD, IgG1, IgG2, IgG3, IgG4, IgE, IgA1 and IgA2); but especially an antibody of the IgG4 isotype; alternatively, in another embodiment, it can be an antigen-binding fragment (e.g., Fab, $F(ab')_2$, and Fv) of a whole antibody.

[0156] The invention thus also relates to antigen-binding fragments of the antibodies described herein. In one embodiment of the invention, the fragment is selected from the group consisting of a Fab fragment, a Fab' fragment, a $F(ab)_2$fragment, and a F_v fragment, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

[0157] In another embodiment, the antibody or antigen-binding fragment of the invention is conjugated to polyethylene glycol. In yet another embodiment, the constant region of the antibody of the invention is modified to reduce at least one constant region-mediated biological effector function relative to an unmodified antibody. In still another embodiment, the antibody or antigen-binding fragment of the invention comprises a Fc region having an altered effector function.

[0158] The invention further relates to a nucleotide molecule comprising a nucleotide sequence encoding a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as disclosed herein before.

[0159] In particular, the invention relates to a nucleotide molecule comprising a nucleotide sequence encoding a stretch of contiguous amino acid molecules as given in SEQ ID NO: 2 and 3, respectively, or the complementary sequence, representing the Complementarity Determining Regions (CDRs) 2 and 3 of the Heavy Chain Variable Region (HCVR).

[0160] More particularly, the invention relates to a nucleotide molecule comprising a nucleotide sequence encoding a stretch of contiguous amino acid molecules as given in SEQ ID NO: 4, or the complementary sequence, representing the Complementarity Determining Regions (CDRs) 1 of the Light Chain Variable Region (LCVR).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6766

[0161] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence as given in SEQ ID NO: 18 and SEQ ID NO: 19, or the complementary sequence, encoding the amino acid sequence of CDR 2 and CDR 3, respectively, of the Heavy Chain Variable Region (HCVR).

[0162] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence as given in SEQ ID NO: 20, or the complementary sequence, encoding the nucleotide sequence of CDR 1 of the Light Chain Variable Region (LCVR).

[0163] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 21, or the complementary sequence, encoding the light chain variable region.

[0164] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 22, or the complementary sequence, encoding the complete light chain variable region including signal sequences.

[0165] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence encoding the light chain variable region of SEQ ID NO: 22 and the light chain constant region of SEQ ID NO: 23. The invention also comprises the complementary strand of said nucleotide molecule.

[0166] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 24 encoding the heavy chain variable region. The invention also comprises the complementary strand of said nucleotide molecule.

[0167] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence of SEQ ID NO: 25 encoding the complete heavy chain variable region including signal sequences. The invention also comprises the complementary strand of said nucleotide molecule.

[0168] In another embodiment of the invention a nucleotide molecule is provided comprising a nucleotide sequence encoding the heavy chain variable region of SEQ ID NO: 25 and the heavy chain constant region of SEQ ID NO: 26. The invention also comprises the complementary strand of said nucleotide molecule.

[0169] Also comprised by the present invention is a nucleotide sequence which hybridizes to one of the above-described antibody-encoding nucleotide sequences of the invention,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6767

particularly to the complementary strand thereof, either in isolation or as part of larger nucleotide molecule.

[0170] In particular, the invention relates to a nucleotide sequence that hybridizes under conventional hybridization conditions, particularly under stringent hybridization conditions, to any of the nucleotide sequences given in SEQ ID NOs: 18-26 and 29 - 32, particularly to the complementary strand thereof.

[0171] In another embodiment of the invention an expression vector is provided comprising the nucleic acid molecule according to the invention and as mentioned herein before.

[0172] In another embodiment of the invention a cell is provided comprising an expression vector comprising the nucleic acid according to the invention and as mentioned herein before.

[0173] In still another embodiment, the invention relates to a composition comprising the antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before including any functionally equivalent antibody or any derivative or functional parts thereof, in a therapeutically effective amount, in particular a composition which is a pharmaceutical or therapeutic composition, optionally further comprising a pharmaceutically acceptable carrier.

[0174] In another embodiment of the invention, said composition comprises the antibody in a therapeutically effective amount.

[0175] Further comprised by the invention is a composition comprising an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before including any functionally equivalent antibody or any derivative or functional parts thereof, in a therapeutically effective amount and, optionally, a further biologically active substance and/or a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

[0176] In particular, the invention relates to a composition or mixture, wherein the further biologically active substance is a compound used in the medication of amyloidoses, a group of diseases and disorders associated with amyloid or amyloid-like protein such as the Aβ protein involved in Alzheimer's disease.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6768

[0177] In another embodiment of the invention, the other biologically active substance or compound may also be a therapeutic agent that may be used in the treatment of amyloidosis caused by amyloid β or may be used in the medication of other neurological disorders.

[0178] The other biologically active substance or compound may exert its biological effect by the same or a similar mechanism as the antibody according to the invention or by an unrelated mechanism of action or by a multiplicity of related and/or unrelated mechanisms of action.

[0179] Generally, the other biologically active compound may include neutron-transmission enhancers, psychotherapeutic drugs, acetylcholine esterase inhibitors, calcium-channel blockers, biogenic amines, benzodiazepine tranquillizers, acetylcholine synthesis, storage or release enhancers, acetylcholine postsynaptic receptor agonists, monoamine oxidase-A or –B inhibitors, N-methyl-D-aspartate glutamate receptor antagonists, non-steroidal anti-inflammatory drugs, antioxidants, and serotonergic receptor antagonists.

[0180] More particularly, the invention relates to a composition or mixture comprising at least one compound selected from the group consisting of compounds effective against oxidative stress, anti-apoptotic compounds, metal chelators, inhibitors of DNA repair such as pirenzepin and metabolites, 3-amino-1-propanesulfonic acid (3APS), 1,3-propanedisulfonate (1,3PDS), α-secretase activators, β- and γ-secretase inhibitors, tau proteins, neurotransmitter, β-sheet breakers, attractants for amyloid beta clearing / depleting cellular components, inhibitors of N-terminal truncated amyloid beta including pyroglutamated amyloid beta 3-42, anti-inflammatory molecules, or cholinesterase inhibitors (ChEIs) such as tacrine, rivastigmine, donepezil, and/or galantamine, M1 agonists and other drugs including any amyloid or tau modifying drug and nutritive supplements, and nutritive supplements, together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

[0181] The invention further relates to a composition or mixture, wherein the compound is a cholinesterase inhibitor (ChEIs), particularly a mixture, wherein the compound is one selected from the group consisting of tacrine, rivastigmine, donepezil, galantamine, niacin and memantine.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6769

[0182] In a further embodiment, the compositions according to the invention may comprise niacin or memantine together with an antibody according to the present invention and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

[0183] In still another embodiment of the invention compositions are provided that comprise "atypical antipsychotics" such as, for example clozapine, ziprasidone, risperidone, aripiprazole or olanzapine for the treatment of positive and negative psychotic symptoms including hallucinations, delusions, thought disorders (manifested by marked incoherence, derailment, tangentiality), and bizarre or disorganized behavior, as well as anhedonia, flattened affect, apathy, and social withdrawal in a subject in need thereof, together with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein and, optionally, a pharmaceutically acceptable carrier and/or a diluent and/or an excipient.

[0184] In a specific embodiment of the invention, the compositions and mixtures according to the invention and as described herein before comprise the antibody and the biologically active substance, respectively, in a therapeutically effective amount.

[0185] Other compounds that can be suitably used in mixtures in combination with the antibody according to the present invention are described in WO 2004/058258 (*see* especially pages 16 and 17) including therapeutic drug targets (page 36-39), alkanesulfonic acids and alkanolsulfuric acids (pages 39-51), cholinesterase inhibitors (pages 51-56), NMDA receptor antagonists (pages 56-58), estrogens (pages 58-59), non-steroidal anti-inflammatory drugs (pages 60-61), antioxidants (pages 61-62), peroxisome proliferators-activated receptor (PPAR) agonists (pages 63-67), cholesterol-lowering agents (pages 68-75); amyloid inhibitors (pages 75-77), amyloid formation inhibitors (pages 77-78), metal chelators (pages 78-79), anti-psychotics and anti-depressants (pages 80-82), nutritional supplements (pages 83-89) and compounds increasing the availability of biologically active substances in the brain (see pages 89-93) and prodrugs (pages 93 and 94), which document is incorporated herein by reference.

[0186] In another embodiment, the invention relates to a composition comprising the antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6770

according to the invention and as described herein before and/or the biologically active substance in a therapeutically effective amount.

[0187] The invention further relates to the use of an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before and/or a functional part thereof and/or a pharmaceutical composition, or a mixture comprising said antibody, for the preparation of a medicament for treating or alleviating the effects of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, in a subject in need thereof.

[0188] Also comprised by the present invention is a method for the preparation of an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before and/or a functional part thereof and/or a pharmaceutical composition, or a mixture comprising said antibody and/or a functional part thereof, particularly in a therapeutically effective amount, for use in a method of preventing, treating or alleviating the effects of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes;

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6771

senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, in a subject in need thereof, comprising formulating an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention in a pharmaceutically acceptable form.

[0189] Further comprised by the present invention is a method for preventing, treating or alleviating the effects of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, in a subject in need thereof by administering an antibody and/or a functional part thereof, but particularly a humanized antibody and/or a functional part thereof, or a composition or mixture comprising such an antibody and/or a functional part thereof, to a subject, including a mammal or a human affected by such a disorder, in a therapeutically effective amount.

[0190] It is also an object of the invention to provide a method for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), particularly a disease or condition characterized by a loss of cognitive memory capacity in a subject in need thereof, by administering to a subject, particularly a mammal or a human, affected by such a disorder an antibody, particularly a pharmaceutical composition according to the invention and as described herein.

[0191] In a specific embodiment the invention provides a method for retaining or increasing cognitive memory capacity but, particularly, for restoring the cognitive memory capacity of a subject, particularly a mammal or a human, suffering from memory impairment by administering to a subject, particularly a mammal or a human, in need thereof an antibody,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6772

particularly a pharmaceutical or therapeutic composition according to the invention and as described herein before.

[0192] It is a further object of the invention to provide a therapeutic composition and a method of producing such a composition as well as a method for the treatment of amyloidosis, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidosis and age-related amyloidosis including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), particularly a disease or condition characterized by a loss of cognitive memory capacity, in a subject in need thereof using an antibody according to the invention and as described herein before.

[0193] In particular, the invention relates to the treatment of a subject, particularly a mammal or a human, suffering from an amyloid-associated condition characterized by a loss of cognitive memory capacity, that leads to the retention of cognitive memory capacity.

[0194] The invention further relates to a method of diagnosis of an amyloid-associated disease or condition in a subject comprising detecting the immunospecific binding of an antibody or an active fragment thereof to an epitope of the amyloid protein in a sample or *in situ* which includes the steps of

(a) bringing the sample or a specific body part or body area of the subject suspected to contain the amyloid protein into contact with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof, which antibody binds an epitope of the amyloid protein;

(b) allowing the antibody and/or a functional part thereof, to bind to the amyloid protein to form an immunological complex;

(c) detecting the formation of the immunological complex; and

(d) correlating the presence or absence of the immunological complex with the presence or absence of amyloid protein in the sample or specific body part or area of the subject.

[0195] Also comprised is a method of determining the extent of amyloidogenic plaque burden in a tissue and/or body fluids of a subject in need thereof comprising

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6773

(a) obtaining a sample representative of the tissue and/or body fluids of the subject under investigation;

(b) testing said sample for the presence of amyloid protein with an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof;

(c) determining the amount of antibody bound to the protein; and

(d) calculating the plaque burden in the tissue and/or body fluids of the subject.

[0196] In particular, the invention relates to a method of determining the extent of amyloidogenic plaque burden in a tissue and/or body fluids of a subject in need thereof, wherein the formation of the immunological complex in step c) is determined such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

[0197] In another embodiment of the invention, a test kit for detection and diagnosis of amyloid-associated diseases and conditions in a subject is provided comprising an antibody, particularly a monoclonal antibody according to the invention, but particularly a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof according to the invention and as described herein before, and/or a functional part thereof.

[0198] In particular, the invention relates to a test kit for detection and diagnosis of amyloid-associated diseases and conditions in a subject in need thereof comprising a container holding one or more antibodies according to the present invention, and/or a functional part thereof, and instructions for using the antibodies for the purpose of binding to amyloid protein to form an immunological complex and detecting the formation of the immunological complex such that presence or absence of the immunological complex correlates with presence or absence of amyloid protein.

[0199] In another aspect, the invention provides methods and compositions for preventing, treating, or detecting a disease associated with amyloidosis in a subject in need thereof using immunoglobulins as described herein that further comprise a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region. The Fc region mediates the effector function of the antibody or fragment thereof. By modulating the ability of the Fc portion of the antibody or fragment thereof to bind to or

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6774

activate its receptor, it is possible to abrogate or enhance the effector function of the antibody or fragment thereof.

[0200] Thus, in another aspect, the invention provides an antibody or fragment thereof of the invention further comprising a variant Fc region comprising at least one amino acid mutation which decreases effector function. In one such aspect, the at least one amino acid mutation decreases glycosylation of the antibody or fragment thereof. In another such aspect, the at least one amino acid mutation decreases binding to a cognate Fc receptor. In another such aspect, the at least one amino acid mutation decreases activation of a cognate Fc receptor upon binding of the antibody or fragment thereof. In one such aspect, the variant Fc region is a variant IgG1 Fc region. In one such aspect, the antibody or fragment thereof comprises a D265A mutation in the Fc region.

[0201] In another aspect, the invention provides an antibody or fragment thereof of the invention further comprising a variant Fc region comprising at least one amino acid mutation which increases effector function. In one such aspect, the at least one amino acid mutation enhances glycosylation of the antibody or fragment thereof. In another such aspect, the at least one amino acid mutation increases binding to a cognate Fc receptor. In another such aspect, the at least one amino acid mutation increases activation of a cognate Fc receptor upon binding of the antibody or fragment thereof. These and other objects, features and advantages of the present invention will become apparent after a review of the following detailed description of the disclosed embodiment and the appended claims.

BRIEF DESCRIPTION OF THE DRAWINGS

[0202] Figure 1 (Example 2): Chimeric antibody heavy chain expression vector

[0203] Figure 2 (Example 2): Chimeric antibody light chain expression vector

[0204] Figure 3 (Example 2): Expression Cassette of the mouse light chain variable region of the Chimeric Antibody

[0205] Figure 4 (Example 2): Expression Cassette of the mouse heavy chain variable region of the Chimeric Antibody

[0206] Figure 5 (Example 5.2): Comparison of the mouse heavy chain variable region to the closest murine germ line sequence

[0207] Figure 6 (Example 8): Activity of purified humanized C2 antibodies

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6775

[0208] Figure 7 (Example 9): Binding activity of antibodies produced by transient expression of C2 modified CDRL2 constructs in conjunction with C2 chimeric heavy chain, compared to chimeric antibody C2ChVHAF/ChVK, produced by transient transfection and purified antibody.

[0209] Figure 8 (Example 11): Results of Immunohistochemical Binding Assay with chimeric antibody AF (IgG4) and humanized antibody H4K1 (IgG4)

[0210] Figure 9 (Example 12): Functionality of mC2 on Amyloid fibers. A) Comparison of ^{13}C CPMAS spectra and fits for U-^{13}C Tyr10 and Val12 labelled amyloid β1-42 fibres incubated with PBS (left served as control) or ACI-7-C2 (right) for 24 hours and then lyophilized. The peak at c33 ppm correspondes to the beta sheet conformation of the fibres whilst the peak at 30 ppm is a result of random coil conformation. B) Comparison of the fitted parameters for the two conformations of Val12 Cβ. The fitted chemical shifts for the two conformations are quite similar but the integral intensities are very different, reflecting a reduction in the original beta sheet conformation by approximately 35% (1-(53.5/81.7)), in agreement with the value obtained from the fluorescent measurement.

[0211] Figure 10 (Example 12): Binding Affinity of humanized C2 in ELISA.

[0212] Figure 11 (Example 14): Conformation-specific binding of mC2 to different classes of Amyloid Protein. Pellet preparation in the legend to this figure refers to $A\beta_{1-42}$ fibers, supernatant preparation refers to amyloid monomers.

[0213] Figure 12: Humanized C2 VK sequences compared to murine sequence and human acceptor sequences DPK15 AND J_K1

[0214] Figure 13: Humanized C2 VH sequences compared to murine sequence and human acceptor sequences DP54 AND J_H6

[0215] Figure 14: Complete DNA and protein sequence of light chain variable region of C2 humanized antibody, C2HuVK1

[0216] Figures 15-1-15-5: Complete DNA and protein sequence of light chain constant region (human C Kappa) of humanized C2 antibody

[0217] Figures 16-1-16-2: Complete DNA and protein sequence of heavy chain constant region (human IgG4 ser228-pro) of humanized C2 antibody

[0218] Figure 17 1-3 (Example 15): Results of Epitope Mapping experiments

[0219] Figure 18 (Example 13): Results of aggregation assay experiments

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6776

[0220] Figure 19 (Example 13): Results of disaggregation assay experiments

[0221] Figure 20: (Example 16): Results of neuroprotection experiments with humanized antibody C2.

BRIEF DESCRIPTION OF THE SEQUENCES

[0222] SEQ ID NO: 1 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR1)

[0223] SEQ ID NO: 2 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR2)

[0224] SEQ ID NO: 3 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region (CDR3)

[0225] SEQ ID NO: 4 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR1)

[0226] SEQ ID NO: 5 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR2)

[0227] SEQ ID NO: 6 Amino acid sequence of C2 HuVK 1 humanized light chain variable region (CDR3)

[0228] SEQ ID NO: 7 Amino acid sequence of Aβ epitope region 2

[0229] SEQ ID NO: 8 Amino acid sequence of Aβ epitope region 1

[0230] SEQ ID NO: 9 Amino acid sequence of Aβ epitope region 2 modified

[0231] SEQ ID NO: 10 Amino acid sequence of Aβ epitope region 1 modified

[0232] SEQ ID NO: 11 Amino acid sequence of Epitope region modified complete

[0233] SEQ ID NO: 12 Amino acid sequence of C2 HuVK 1 humanized light chain variable region

[0234] SEY ID NO: 13 Amino acid sequence of C2 humanized light chain

[0235] SEQ ID NO: 14 Amino acid sequence of humanized C2 light chain constant region

[0236] SEQ ID NO: 15 Amino acid sequence of C2 HuVH AF 4 humanized heavy chain variable region

[0237] SEQ ID NO: 16 Amino acid sequence of C2 humanized heavy chain

[0238] SEQ ID NO: 17 Amino acid sequence of IG GAMMA-4 CHAIN C REGION – modified

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6777

[0239] SEQ ID NO: 18 Nucleotide sequence of CDR2 of C2 HuVH AF 4 humanised heavy chain variable region

[0240] SEQ ID NO: 19 Nucleotide sequence of CDR3 of C2 HuVH AF 4 humanised heavy chain variable region

[0241] SEQ ID NO: 20 Nucleotide sequence of CDR1 of C2 HuVK 1 humanised light chain variable region

[0242] SEQ ID NO: 21 Nucleotide sequence of C2 HuVK 1 humanized light chain variable region

[0243] SEQ ID NO: 22 Nucleotide sequence of C2 humanized light chain

[0244] SEQ ID NO: 23 Nucleotide sequence of C2 humanized light chain constant region

[0245] SEQ ID NO: 24 Nucleotide sequence of C2 HuVH AF 4 humanized heavy chain variable region

[0246] SEQ ID NO: 25 Nucleotide sequence of C2 humanized heavy chain

[0247] SEQ ID NO: 26 Nucleotide sequence of C2 humanized heavy chain constant region

[0248] SEQ ID NO: 27 Amino acid sequence of Mouse C2 Light Chain Variable Region

[0249] SEQ ID NO: 28 Amino acid sequence of Mouse C2 Heavy Chain Variable Region

[0250] SEQ ID NO: 29 Nucleotide sequence of Mouse C2 Light Chain Variable Region

[0251] SEQ ID NO: 30 Nucleotide sequence of Mouse C2 Light Chain

[0252] SEQ ID NO: 31 Nucleotide sequence of Mouse C2 Heavy Chain Variable Region

[0253] SEQ ID NO: 32 Nucleotide sequence of Mouse C2 Heavy Chain

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

[0254] The terms "polypeptide", "peptide", and "protein", as used herein, are interchangeable and are defined to mean a biomolecule composed of amino acids linked by a peptide bond.

[0255] The terms "a", "an" and "the" as used herein are defined to mean "one or more" and include the plural unless the context is inappropriate.

[0256] The language "diseases and disorders which are caused by or associated with amyloid or amyloid-like proteins" includes, but is not limited to, diseases and disorders caused by the presence or activity of amyloid-like proteins in monomeric, fibril, or polymeric

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6778

state, or any combination of the three. Such diseases and disorders include, but are not limited to, amyloidosis, endocrine tumors, and macular degeneration.

[0257] The term "amyloidosis" refers to a group of diseases and disorders associated with amyloid plaque formation including, but not limited to, secondary amyloidosis and age-related amyloidosis such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), including diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), Adult Onset Diabetes; senile cardiac amyloidosis, as well as eye diseases including macular degeneration, drusen-related optic neuropathy, and catatact due to beta-amyloid deposition.

[0258] The terms "detecting" or "detected" as used herein mean using known techniques for detection of biologic molecules such as immunochemical or histological methods and refer to qualitatively or quantitatively determining the presence or concentration of the biomolecule under investigation.

[0259] "Polymeric soluble amyloid" refers to multiple aggregated monomers of amyloid peptides, or of amyloid-like peptides, or of modified or truncated amyloid peptides or of other derivates of amyloid peptides forming oligomeric or polymeric structures which are soluble in the mammalian or human body more particularly in the brain, but particularly to multiple aggregated monomers of amyloid β (Aβ) or of modified or truncated amyloid β (Aβ) peptides or of derivatives thereof, which are soluble in the mammalian or human body more particularly in the brain.

[0260] "Amyloid β, Aβ or β-amyloid" is an art recognized term and refers to amyloid β proteins and peptides, amyloid β precursor protein (APP), as well as modifications, fragments and any functional equivalents thereof. In particular, by amyloid β as used herein is meant any fragment produced by proteolytic cleavage of APP but especially those fragments which are involved in or associated with the amyloid pathologies including, but not limited to, $A\beta_{1-38}$, $A\beta_{1-39}$, $A\beta_{1-40}$, $A\beta_{1-41}$ $A\beta_{1-42}$ and $A\beta_{1-43}$.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6779

[0261] The structure and sequences of the amyloid β peptides as mentioned above are well known to those skilled in the art and methods of producing said peptides or of extracting them from brain and other tissues are described, for example, in Glenner and Wong, Biochem Biophys Res Comm129, 885-890 (1984). Moreover, amyloid β peptides are also commercially available in various forms.

[0262] By "isolated" is meant a biological molecule free from at least some of the components with which it naturally occurs.

[0263] The terms "antibody" or "antibodies" as used herein are art-recognized terms and are understood to refer to molecules or active fragments of molecules that bind to known antigens, particularly to immunoglobulin molecules and to immunologically active portions of immunoglobulin molecules, i.e molecules that contain a binding site that specifically binds an antigen. An immunoglobulin is a protein comprising one or more polypeptides-substantially encoded by the immunoglobulin kappa and lambda, alpha, gamma, delta, epsilon and mu constant region genes, as well as myriad immunoglobulin variable region genes. Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, which in turn define the immunoglobulin classes, IgG, IgM, IgA, IgD and IgE, respectively. Also subclasses of the heavy chain are known. For example, IgG heavy chains in humans can be any of IgG1, IgG2, IgG3 and IgG4 subclass. The immunoglobulin according to the invention can be of any class (IgG, IgM, IgD, IgE, IgA and IgY) or subclass (IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) of immunoglobulin molecule.

[0264] As used herein "specifically binds" in reference to an antibody means that the antibody binds to its target antigen with greater affinity that it does to a structurally different antigen(s).

[0265] A typical immunoglobulin structural unit is known to comprise a tetramer. Each tetramer is composed of two identical pairs of polypeptide chains, each pair having one "light" (about 25 kD) and one "heavy" chain (about 50-70 kD). The N-terminus of each chain defines a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. The terms variable light chain (V_L) and variable heavy chain (V_H) refer to these light and heavy chains respectively.

[0266] Antibodies exist as full length intact antibodies or as a number of well-characterized fragments produced by digestion with various peptidases or chemicals. Thus, for example,

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6780

pepsin digests an antibody below the disulfide linkages in the hinge region to produce $F(ab')_2$, a dimer of Fab which itself is a light chain joined to V_H-CH_1 by a disulfide bond. The $F(ab')_2$ may be reduced under mild conditions to break the disulfide linkage in the hinge region thereby converting the $F(ab')_2$ dimer into an Fab' monomer. The Fab' monomer is essentially a Fab fragment with part of the hinge region (see, Fundamental Immunology, W. E. Paul, ed., Raven Press, N.Y. (1993), for a more detailed description of other antibody fragments). While various antibody fragments are defined in terms of the digestion of an intact antibody, one of skill will appreciate that any of a variety of antibody fragments may be synthesized de novo either chemically or by utilizing recombinant DNA methodology. Thus, the term antibody, as used herein also includes antibody fragments either produced by the modification of whole antibodies or synthesized de novo or antibodies and fragments obtained by using recombinant DNA methodologies.

[0267] "Antibodies" are intended within the scope of the present invention to include monoclonal antibodies, polyclonal antibodies, chimeric, single chain, bispecific, simianized, human and humanized antibodies as well as active fragments thereof. Examples of active fragments of molecules that bind to known antigens include separated light and heavy chains, Fab, Fab/c, Fv, Fab', and $F(ab')_2$ fragments, including the products of an Fab immunoglobulin expression library and epitope-binding fragments of any of the antibodies and fragments mentioned above.

[0268] These active fragments can be derived from an antibody of the present invention by a number of techniques. For example, monoclonal antibodies can be cleaved with an enzyme, such as pepsin, and subjected to HPLC gel filtration. The appropriate fraction containing Fab fragments can then be collected and concentrated by membrane filtration and the like. For further description of general techniques for the isolation of active fragments of antibodies, see for example, Khaw, B. A. et al. J. Nucl. Med. 23:1011-1019 (1982); Rousseaux et al. Methods Enzymology, 121:663-69, Academic Press, 1986.

[0269] Recombinantly made antibodies may be conventional full length antibodies, active antibody fragments known from proteolytic digestion, unique active antibody fragments such as Fv or single chain Fv (scFv), domain deleted antibodies, and the like. An Fv antibody is about 50 Kd in size and comprises the variable regions of the light and heavy chain. A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which may be

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6781

expressed from a nucleic acid including VH- and VL-encoding sequences either joined directly or joined by a peptide-encoding linker. See Huston, et al. (1988) Proc. Nat. Acad. Sci. USA, 85:5879-5883. A number of structures for converting the naturally aggregated, but chemically separated light and heavy polypeptide chains from an antibody V region into an scFv molecule which will fold into a three dimensional structure substantially similar to the structure of an antigen-binding site. See, e.g. U.S. Patent Nos. 5,091,513, 5,132,405 and 4,956,778.

[0270] The combining site refers to the part of an antibody molecule that participates in antigen binding. The antigen binding site is formed by amino acid residues of the N-terminal variable ("V") regions of the heavy ("H") and light ("L") chains. The antibody variable regions comprise three highly divergent stretches referred to as "hypervariable regions" or "complementarity determining regions" (CDRs) which are interposed between more conserved flanking stretches known as "framework regions" (FRs). In an antibody molecule, the three hypervariable regions of a light chain (LCDR1, LCDR2, and LCDR3) and the three hypervariable regions of a heavy chain (HCDR1, HCDR2 and HCDR3) are disposed relative to each other in three dimensional space to form an antigen binding surface or pocket. The antibody combining site therefore represents the amino acids that make up the CDRs of an antibody and any framework residues that make up the binding site pocket.

[0271] The identity of the amino acid residues in a particular antibody that make up the combining site can be determined using methods well known in the art. For example, antibody CDRs may be identified as the hypervariable regions originally defined by Kabat et al. (see, "Sequences of Proteins of Immunological Interest," E. Kabat et al., U.S. Department of Health and Human Services; Johnson, G and Wu, TT (2001) Kabat Database and its applications: future directions. Nucleic Acids Research, 29: 205-206; http://immuno.bme.nwa.edu). The positions of the CDRs may also be identified as the structural loop structures originally described by Chothia and others, (see Chothia and Lesk, J. Mol. Biol. 196, 901 (1987), Chothia et al., Nature 342, 877 (1989), and Tramontano et al., J. Mol. Biol. 215, 175 (1990)). Other methods include the "AbM definition" which is a compromise between Kabat and Chothia and is derived using Oxford Molecular's AbM antibody modeling software (now Accelrys) or the "contact definition" of CDRs by Macallum et al., ("Antibody-antigen interactions: contact analysis and binding site

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6782

topography," J Mol Biol. 1996 Oct 11;262(5):732-45). The following chart identifies CDRs based upon various known definitions.

Loop	Kabat	AbM	Chothia	Contact
L1	L24 -- L34	L24 -- L34	L24 -- L34	L30 -- L36
L2	L50 -- L56	L50 -- L56	L50 -- L56	L46 -- L55
L3	L89 -- L97	L89 -- L97	L89 -- L97	L89 -- L96
H1	H31 -- H35B	H26 -- H35B	H26 -- H32..34	H30 -- H35B
(Kabat Numbering)				
H1	H31 -- H35	H26 -- H35	H26 -- H32	H30 -- H35
(Chothia Numbering)				
H2	H50 -- H65	H50 -- H58	H52 -- H56	H47 -- H58
H3	H95 -- H102	H95 -- H102	H95 -- H102	H93 -- H101

[0272] General guidelines by which one may identify the CDRs in an antibody from sequence alone are as follows:

LCDR1:

Start - Approximately residue 24.

Residue before is always a Cys.

Residue after is always a Trp. Typically TRP is followed with TYR-GLN, but also may be followed by LEU-GLN, PHE-GLN, or TYR-LEU.

Length is 10 to 17 residues.

LCDR2:

Start - 16 residues after the end of L1.

Sequence before is generally ILE-TYR, but also may be VAL-TYR, ILE-LYS, or ILE-PHE.

Length is generally 7 residues.

LCDR3:

Start – generally 33 residues after end of L2.

Residue before is a Cys.

Sequence after is PHE-GLY-X-GLY.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6783

Length is 7 to 11 residues.

HCDR1:

Start – at approximately residue 26 (four residues after a CYS) [Chothia / AbM definition]

Kabat definition starts 5 residues later.

Sequence before is CYS-X-X-X.

Residues after is a TRP, typically followed by VAL, but also followed by ILE, or ALA.

Length is 10 to 12 residues under AbM definition while Chothia definition excludes the last 4 residues.

HCDR2:

Start - 15 residues after the end of Kabat /AbM definition of CDR-H1.

Sequence before typically LEU-GLU-TRP-ILE-GLY (SEQ ID NO. 1), but a number of variations are possible.

Sequence after is LYS/ARG-LEU/ILE/VAL/PHE/THR/ALA-THR/SER/ILE/ALA

Length is 16 to 19 residues under Kabat definition (AbM definition ends 7 residues earlier).

HCDR3:

Start –33 residues after end of CDR-H2 (two residues after a CYS).

Sequence before is CYS-X-X (typically CYS-ALA-ARG).

Sequence after is TRP-GLY-X-GLY.

Length is 3 to 25 residues.

[0273] The identity of the amino acid residues in a particular antibody that are outside the CDRs, but nonetheless make up part of the combining site by having a side chain that is part of the lining of the combining site (i.e., it is available to linkage through the combining site), can be determined using methods well known in the art such as molecular modeling and X-ray crystallography. See e.g., Riechmann et al., (1988) Nature, 332:;323-327.

[0274] Chimeric antibodies are those in which one or more regions of the antibody are from one species of animal and one or more regions of the antibody are from a different species of animal. A preferred chimeric antibody is one which includes regions from a primate immunoglobulin. A chimeric antibody for human clinical use is typically understood to have variable regions from a non-human animal, e.g. a rodent, with the constant regions from a human. In contrast, a humanized antibody uses CDRs from the non-human antibody with most or all of the variable framework regions from and all the constant regions from a

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6784

human immunoglobulin. A human chimeric antibody is typically understood to have the variable regions from a rodent. A typical human chimeric antibody has human heavy constant regions and human light chain constant regions with the variable regions of both the heavy and light coming from a rodent antibody. A chimeric antibody may include some changes to a native amino acid sequence of the human constant regions and the native rodent variable region sequence. Chimeric and humanized antibodies may be prepared by methods well known in the art including CDR grafting approaches (see, e.g., U.S. Patent Nos. 5,843,708; 6,180,370; 5,693,762; 5,585,089; 5,530,101), chain shuffling strategies (see e.g., U.S. Patent No. 5,565,332; Rader et al., Proc. Natl. Acad. Sci. USA (1998) 95:8910-8915), molecular modeling strategies (U.S. Patent No. 5,639,641), and the like.

[0275] A "humanized antibody" as used herein in the case of a two chain antibody is one where at least one chain is humanized. A humanized antibody chain has a variable region where one or more of the framework regions are human. A humanized antibody which is a single chain is one where the chain has a variable region where one or more of the framework regions are human. The non-human portions of the variable region of the humanized antibody chain or fragment thereof is derived from a non-human source, particularly a non-human antibody, typically of rodent origin. The non-human contribution to the humanized antibody is typically provided in form at least one CDR region which is interspersed among framework regions derived from one (or more) human immunoglobulin(s). In addition, framework support residues may be altered to preserve binding affinity.

[0276] The humanized antibody may further comprise constant regions (*e.g.*, at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the case of a heavy chain). The constant regions of a humanized antibody if present generally are human.

[0277] Methods to obtain "humanized antibodies" are well known to those skilled in the art. (see, e.g., Queen et al., Proc. Natl Acad Sci USA, 86:10029-10032 (1989), Hodgson et al., Bio/Technology, 9:421 (1991)).

[0278] A "humanized antibody" may also be obtained by a novel genetic engineering approach that enables production of affinity-matured human-like polyclonal antibodies in large animals such as, for example, rabbits and mice. See, e.g. U.S. Pat No. 6,632,976.

[* sections here replaced with definitions above]

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6785

[0279] The term constant region (CR) as used herein refers to constant regions genes of the immunoglobulin. The constant region genes encode the portion of the antibody molecule which confers effector functions. For Chimeric human antibodies and humanized antibodies, typically non-human (*e.g.*, murine), constant regions are substituted by human constant regions. The constant regions of the subject chimeric or humanized antibodies are typically derived from human immunoglobulins. The heavy chain constant region can be selected from any of the five isotypes: alpha, delta, epsilon, gamma or mu. Further, heavy chains of various subclasses (such as the IgG subclasses of heavy chains) are responsible for different effector functions and thus, by choosing the desired heavy chain constant region, antibodies with desired effector function can be produced. Constant regions that may be used within the scope of this invention are gamma 1 (IgGl), particularly an Fc region of the gamma 1 (IgG1) isotype, gamma 3 (IgG3) and especially gamma 4 (IgG4). The light chain constant region can be of the kappa or lambda type, preferably of the kappa type. In one embodiment the light chain constant region is the human kappa constant chain (Heiter *et al.* (1980) *Cell* 22:197-207) and the heavy constant chain is the human IgG4 constant chain.

[0280] The term "Fc region" is used to define a C-terminal region of an immunoglobulin heavy chain. The "Fc region" may be a native sequence Fc region or a variant Fc region. Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The Fc region of an immunoglobulin generally comprises two constant domains, CH2 and CH3.

[0281] A "functional Fc region" possesses an "effector function" of a native sequence Fc region. Exemplary "effector functions" include C1q binding; complement dependent cytotoxicity; Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor; BCR), etc. Such effector functions generally require the Fc region to be combined with a binding domain (*e.g.* an antibody variable domain) and can be assessed using various assays as herein disclosed, for example. A functional Fc region generally includes two heavy chain CH2 and CH3 containing polypeptides which are in association.

[0282] A "native sequence Fc region" comprises an amino add sequence identical to the amino acid sequence of an Fc region found in nature and naturally occurring variants thereof.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6786

[0283] A "variant Fc region" comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one "amino acid modification" as herein defined. Preferably, the variant Fc region has at least one amino acid substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, *e.g.* from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

[0284] An "amino acid modification" refers to a change in the amino acid sequence of a predetermined amino acid sequence. Exemplary modifications include an amino acid substitution, insertion and/or deletion. The preferred amino acid modification herein is a substitution.

[0285] An "amino acid modification at" a specified position, *e.g.* of the Fc region, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. By insertion "adjacent" a specified residue is meant insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue.

[0286] An "amino acid substitution" refers to the replacement of at least one existing amino acid residue in a predetermined amino acid sequence with another different "replacement" amino acid residue. The replacement residue or residues may be "naturally occurring amino acid residues" (*i.e.* encoded by the genetic code) and selected from the group consisting of: alanine (Ala); arginine (Arg); asparagine (Asn); aspartic acid (Asp); cysteine (Cys); glutamine (Gln); glutamic acid (Glu); glycine (Gly); histidine (His); Isoleucine (Ile): leucine (Leu); lysine (Lys); methionine (Met); phenylalanine (Phe); proline (Pro): serine (Ser); threonine (Thr); tryptophan (Trp); tyrosine (Tyr); and valine (Val). Preferably, the replacement residue is not cysteine. Substitution with one or more non-naturally occurring amino acid residues is also encompassed by the definition of an amino acid substitution herein.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6787

[0287] A "non-naturally occurring amino acid residue" refers to a residue, other than those naturally occurring amino acid residues listed above, which is able to covalently bind adjacent amino acid residues(s) in a polypeptide chain. Examples of non-naturally occurring amino acid residues include norleucine, omithine, norvaline, homoserine and other amino acid residue analogues such as those described in Ellman et al. Meth. Enzym. 202:301-336 (1991). To generate such non-naturally occurring amino acid residues, the procedures of Noren et al. Science 244:182 (1989) and Ellman et al., supra, can be used. Briefly, these procedures involve chemically activating a suppressor tRNA with a non-naturally occurring amino acid residue followed by in vitro transcription and translation of the RNA.

[0288] An "amino acid insertions" refers to the incorporation of at least one amino acid into a predetermined amino acid sequence. While the insertion will usually consist of the insertion of one or two amino acid residues, the present application contemplates larger "peptide insertions," *e.g.* insertion of about three to about five or even up to about ten amino acid residues. The inserted residue(s) may be naturally occurring or non-naturally occurring as disclosed above.

[0289] An "amino acid deletion" refers to the removal of at least one amino acid residue from a predetermined amino acid sequence.

[0290] "Antibody-dependent cell-mediated cytotoxicity" or "ADCC" refers to a form of cytotoxicity in which secreted antiobody bound to target antigen expressed by target cells is recognized by Fc receptors (FcRs) present on certain cytotoxic cells (e.g. Natural Killer (NK) cells, neutrophils, and macrophages) enabling these cytotoxic effector cells to bind specifically to recognize the antibody coated target cell and subsequently kill the target cell with cytotoxins. The primary cells for mediating ADCC, NK cells, express Fc.gamma.RIII only, whereas monocytes express Fc.gamma.RI, Fc.gamma.RII and Fc.gamma.RIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol 9:457-92 (1991). To assess ADCC activity of a molecule of interest, an in vitro ADCC assay, such as that described in U.S. Pat. Nos. 5,500,362 or 5,821,337 may be performed. Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, e.g., in a animal model such as that disclosed in Clynes et al. PNAS (USA) 95:652-656 (1998).

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6788

[0291] "Immune effector cells" are leukocytes which express one or more FcRs and perform effector functions. Preferably, the cells express at least Fc.gamma.RIII and perform ADCC effector function. Examples of human leukocytes which mediate ADCC include peripheral blood mononuclear cells (PBMC), natural killer (NK) cells, monocytes, cytotoxic T cells and neutrophils; with PBMCs and NK cells being preferred. The effector cells may be isolated from a native source thereof, e.g. from blood or PBMCs as described herein.

[0292] "Complement dependent cytotoxicity" or "CDC" refers to complement dependent lysis of a target cell which has been bound by antibody reactive with antigen expressed by the target cell. Activation of the classical complement pathway is initiated by the binding of the first component of the complement system (C1q) to antibodies (of the appropriate subclass) which are bound to their cognate antigen. To assess complement activation, a CDC assay, e.g. as described in Gazzano-Santoro et al., J. Immunol. Methods 202:163 (1996), may be performed.

[0293] A polypeptide with a variant IgG Fc with "altered" FcR binding affinity or ADCC activity is one which has either enhanced or reduced FcR binding activity (Fc.gamma.R or FcRn) and/or ADCC activity compared to a parent polypeptide or to a polypeptide comprising a native sequence Fc region. The variant Fc which "exhibits increased binding" to an FcR binds at least one FcR with better affinity than the parent polypeptide. The improvement in binding compared to a parent polypeptide may be about 3 fold, preferably about 5, 10, 25, 50, 60, 100, 150, 200, up to 500 fold, or about 25% to 1000% improvement in binding. The polypeptide variant which "exhibits decreased binding" to an FcR, binds at least one FcR with worse affinity than a parent polypeptide. The decrease in binding compared to a parent polypeptide may be about 40% or more decrease in binding. Such Fc variants which display decreased binding to an FcR may possess little or no appreciable binding to an FcR, e.g., 0-20% binding to the FcR compared to a native sequence IgG Fc region, e.g. as determined in the Examples herein.

[0294] The polypeptide comprising a variant Fc region which "exhibits increased ADCC" or mediates antibody-dependent cell-mediated cytotoxicity (ADCC) in the presence of human effector cells more effectively than a polypeptide having wild type IgG Fc is one which in vitro or in vivo is substantially more effective at mediating ADCC, when the amounts of polypeptide with variant Fc region and the polypeptide with wild type Fc region used in the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6789

assay are essentially the same (all other factors being equal). Generally, such variants will be identified using an in vitro ADCC assay, but other assays or methods for determining ADCC activity, e.g. in an animal model etc, are contemplated. The preferred variant is from about 5 fold to about 100 fold, e.g. from about 25 to about 50 fold, more effective at mediating ADCC than the wild type Fc.

[0295] The term "monoclonal antibody" is also well recognized in the art and refers to an antibody that is the product of a single cloned antibody producing cell. Monoclonal antibodies are typically made by fusing a normally short-lived, antibody-producing B cell to a fast-growing cell, such as a cancer cell (sometimes referred to as an "immortal" cell). The resulting hybrid cell, or hybridoma, multiplies rapidly, creating a clone that produces the antibody.

[0296] For the purpose of the present invention, "monoclonal antibody" is also to be understood to comprise antibodies that are produced by a mother clone which has not yet reached full monoclonality.

[0297] "Functionally equivalent antibody" is understood within the scope of the present invention to refer to an antibody which substantially shares at least one major functional property with an antibody mentioned above and herein described comprising: binding specificity to the β-amyloid protein, particularly to the $A\beta_{1-42}$ protein, and more particularly to the 16-21 epitope region of the $A\beta_{1-42}$ protein, immunoreactivity *in vitro*, inhibition of aggregation of the $A\beta_{1-42}$ monomers into high molecular polymeric fibrils and/or disaggregation of preformed $A\beta_{1-42}$ polymeric fibrils, and/or a β-sheet breaking property and alleviating the effects of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, when administered prophylactically or therapeutically. The antibodies can be of any class such as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6790

IgG, IgM, or IgA, etc or any subclass such as IgG1, IgG2a, etc and other subclasses mentioned herein above or known in the art, but particularly of the IgG4 class. Further, the antibodies can be produced by any method, such as phage display, or produced in any organism or cell line, including bacteria, insect, mammal or other type of cell or cell line which produces antibodies with desired characteristics, such as humanized antibodies. The antibodies can also be formed by combining a Fab portion and an Fc region from different species.

[0298] The term "hybridize" as used refers to conventional hybridization conditions, preferably to hybridization conditions at which 5xSSPE, 1% SDS, 1xDenhardts solution is used as a solution and/or hybridization temperatures are between 35°C and 70°C, preferably 65°C. After hybridization, washing is preferably carried out first with 2xSSC, 1% SDS and subsequently with 0.2xSSC at temperatures between 35°C and 70°C, preferably at 65°C (regarding the definition of SSPE, SSC and Denhardts solution see Sambrook et al. loc. cit.). Stringent hybridization conditions as for instance described in Sambrook et al, supra, are particularly preferred. Particularly preferred stringent hybridization conditions are for instance present if hybridization and washing occur at 65°C as indicated above. Non-stringent hybridization conditions, for instance with hybridization and washing carried out at 45°C are less preferred and at 35°C even less.

[0299] "Homology" between two sequences is determined by sequence identity. If two sequences which are to be compared with each other differ in length, sequence identity preferably relates to the percentage of the nucleotide residues of the shorter sequence which are identical with the nucleotide residues of the longer sequence. Sequence identity can be determined conventionally with the use of computer programs such as the Bestfit program (Wisconsin Sequence Analysis Package, Version 8 for Unix, Genetics Computer Group, University Research Park, 575 Science Drive Madison, WI 53711). Bestfit utilizes the local homology algorithm of Smith and Waterman, Advances in Applied Mathematics 2 (1981), 482-489, in order to find the segment having the highest sequence identity between two sequences. When using Bestfit or another sequence alignment program to determine whether a particular sequence has for instance 95% identity with a reference sequence of the present invention, the parameters are preferably so adjusted that the percentage of identity is calculated over the entire length of the reference sequence and that homology gaps of up to

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6791

5% of the total number of the nucleotides in the reference sequence are permitted. When using Bestfit, the so-called optional parameters are preferably left at their preset ("default") values. The deviations appearing in the comparison between a given sequence and the above-described sequences of the invention may be caused for instance by addition, deletion, substitution, insertion or recombination. Such a sequence comparison can preferably also be carried out with the program "fasta20u66" (version 2.0u66, September 1998 by William R. Pearson and the University of Virginia; see also W.R. Pearson (1990), Methods in Enzymology 183, 63-98, appended examples and http://workbench.sdsc.edu/). For this purpose, the "default" parameter settings may be used.

[0300] The antibody according to the invention may be an immunoglobulin or antibody, which is understood to have each of its binding sites identical (if multivalent) or, in the alternative, may be a "bispecific" or "bifunctional antibody".

[0301] A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

[0302] The term "fragment" refers 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. Fragments can be obtained *via* chemical or enzymatic treatment of an intact or complete antibody or antibody chain. Fragments can also be obtained by recombinant means. Exemplary fragments include Fab, Fab', F(ab')2, Fabc and/or Fv fragments. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that binds antigen or competes with intact antibody (*i.e.*, with the intact antibody from which they were derived) for antigen binding (*i.e.*, specific binding).

[0303] Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', $F(ab')_2$, Fabc, Fv, single chains, and single-chain antibodies.

[0304] "**Fragment**" also refers to a peptide or polypeptide comprising an amino acid sequence of at least 5 contiguous amino acid residues, at least 10 contiguous amino acid residues, at least 15 contiguous amino acid residues, at least 20 contiguous amino acid

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6792

residues, at least 25 contiguous amino acid residues, at least 40 contiguous amino acid residues, at least 50 contiguous amino acid residues, at least 60 contiguous amino residues, at least 70 contiguous amino acid residues, at least contiguous 80 amino acid residues, at least contiguous 90 amino acid residues, at least contiguous 100 amino acid residues, at least contiguous 125 amino acid residues, at least 150 contiguous amino acid residues, at least contiguous 175 amino acid residues, at least contiguous 200 amino acid residues, or at least contiguous 250 amino acid residues of the amino acid sequence of another polypeptide. In a specific embodiment, a fragment of a polypeptide retains at least one function of the polypeptide.

[0305] The term "antigen" refers to an entity or fragment thereof which can bind to an antibody. An immunogen refers to an antigen which can elicit an immune response in an organism, particularly an animal, more particularly a mammal including a human. The term antigen includes regions known as antigenic determinants or epitopes which refers to a portion of the antigen (which are contacted or which play a significant role in supporting a contact reside in the antigen responsible for antigenicity or antigenic determinants.

[0306] As used herein, the term "soluble" means partially or completely dissolved in an aqueous solution.

[0307] Also as used herein, the term "immunogenic" refers to substances which elicit the production of antibodies, T-cells and other reactive immune cells directed against an antigen of the immunogen.

[0308] An immune response occurs when an individual produces sufficient antibodies, T-cells and other reactive immune cells against administered immunogenic compositions of the present invention to moderate or alleviate the disorder to be treated.

[0309] The term immunogenicity as used herein refers to a measure of the ability of an antigen to elicit an immune response (humoral or cellular) when administered to a recipient. The present invention is concerned with approaches that reduce the immunogenicity of the subject human chimeric or humanized antibodies.

[0310] Humanized antibody of reduced immunogenicity refers to a humanized antibody exhibiting reduced immunogenicity relative to the parent antibody, e.g., the murine antibody.

[0311] Humanized antibody substantially retaining the binding properties of the parent antibody refers to a humanized antibody which retains the ability to specifically bind the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6793

antigen recognized by the parent antibody used to produce such humanized antibody. Preferably the humanized antibody will exhibit the same or substantially the same antigen-binding affinity and avidity as the parent antibody. Ideally, the affinity of the antibody will not be less than 10% of the parent antibody affinity, more preferably not less than about 30%, and most preferably the affinity will not be less than 50% of the parent antibody. Methods for assaying antigen-binding affinity are well known in the art and include half-maximal binding assays, competition assays, and Scatchard analysis. Suitable antigen binding assays are described in this application.

[0312] A "back mutation" is a mutation introduced in a nucleotide sequence which encodes a humanized antibody, the mutation results in an amino acid corresponding to an amino acid in the parent antibody (*e.g.*, donor antibody, for example, a murine antibody). Certain framework residues from the parent antibody may be retained during the humanization of the antibodies of the invention in order to substantially retain the binding properties of the parent antibody, while at the same time minimizing the potential immunogenicity of the resultant antibody. In one embodiment of the invention, the parent antibody is of mouse origin. For example, the back mutation changes a human framework residue to a parent murine residue. Examples of framework residues that may be back mutated include, but are not limited to, canonical residues, interface packing residues, unusual parent residues which are close to the binding site, residues in the "Vernier Zone" (which forms a platform on which the CDRs rest) (Foote & Winter, 1992, *J. Mol. Biol.* 224, 487-499), and those close to CDR H3.

[0313] As used herein a "conservative change" refers to alterations that are substantially conformationally or antigenically neutral, producing minimal changes in the tertiary structure of the mutant polypeptides, or producing minimal changes in the antigenic determinants of the mutant polypeptides, respectively, as compared to the native protein. When referring to the antibodies and antibody fragments of the invention, a conservative change means an amino acid substitution that does not render the antibody incapable of binding to the subject receptor. Those of ordinary skill in the art will be able to predict which amino acid substitutions can be made while maintaining a high probability of being conformationally and antigenically neutral. Such guidance is provided, for example in Berzofsky, (1985) *Science* 229:932-940 and Bowie *et al.* (1990) *Science* 247:1306-1310. Factors to be considered that affect the probability of maintaining conformational and antigenic neutrality include, but are

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6794

not limited to: (a) substitution of hydrophobic amino acids is less likely to affect antigenicity because hydrophobic residues are more likely to be located in a protein's interior; (b) substitution of physiochemically similar, amino acids is less likely to affect conformation because the substituted amino acid structurally mimics the native amino acid; and (c) alteration of evolutionarily conserved sequences is likely to adversely affect conformation as such conservation suggests that the amino acid sequences may have functional importance. One of ordinary skill in the art will be able to assess alterations in protein conformation using well-known assays, such as, but not limited to microcomplement fixation methods (Wasserman *et al.* (1961) J. Immunol. 87:290-295; Levine *et al.* (1967) Meth. Enzymol. 11:928-936) and through binding studies using conformation-dependent monoclonal antibodies (Lewis *et al.* (1983) Biochem. 22:948-954).

[0314] Further, the term "therapeutically effective amount" refers to the amount of antibody which, when administered to a human or animal, which is sufficient to result in a therapeutic effect in said human or animal. The effective amount is readily determined by one of skill in the art following routine procedures.

[0315] As used herein, the terms "**treat**," "**prevent**," "**preventing**," and "**prevention**" refer to the prevention of the recurrence or onset of one or more symptoms of a disorder in a subject resulting from the administration of a prophylactic or therapeutic agent.

Construction of Humanized Antibodies

[0316] The present invention may be understood more readily by reference to the following detailed description of specific embodiments included herein. Although the present invention has been described with reference to specific details of certain embodiments, thereof, it is not intended that such details should be regarded as limitations upon the scope of the invention.

[0317] The present invention provides novel methods and compositions comprising highly specific and highly effective antibodies having the ability to specifically recognize and bind to specific epitopes from a range of β-amyloid antigens. The antibodies enabled by the teaching of the present invention are particularly useful for the treatment of amyloidoses, a group of diseases and disorders associated with amyloid plaque formation including secondary amyloidoses and age-related amyloidoses including, but not limited to, neurological disorders such as Alzheimer's Disease (AD), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type); the Guam

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6795

Parkinson-Dementia complex; as well as other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis; Creutzfeld Jacob disease, hereditary cerebral hemorrhage with amyloidosis Dutch type, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), Adult Onset Diabetes; senile cardiac amyloidosis; endocrine tumors, and others, including macular degeneration, to name just a few.

[0318] A fully humanized or reshaped variable region according to the present invention may be created within the scope of the invention by first designing a variable region amino acid sequence that contains non-human-, particularly rodent-derived CDRs, but especially CDRs derived from murine antibody ACI-01-Ab7C2 (named "mC2" throughout the application and deposited 01 December 2005 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Branuschweig, under the provisions of the Budapest Treaty and given accession no DSM ACC2750) embedded in human-derived framework sequences. The non-human-, particularly the rodent-derived CDRs, which may be obtained from the antibody according to the present invention, provide the desired specificity. Accordingly, these residues are to be included in the design of the reshaped variable region essentially unchanged. Any modifications should thus be restricted to a minimum and closely watched for changes in the specificity and affinity of the antibody. On the other hand, framework residues in theory can be derived from any human variable region.

[0319] In order to create a reshaped antibody which shows an acceptable or an even improved affinity, a human framework sequences should be chosen, which is equally suitable for creating a reshaped variable region and for retaining antibody affinity.

[0320] In order to achieve this goal, the best-fit strategy was developed. As it is known that the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding, this strategy aims at minimizing changes that may negatively effect the three-dimensional structure of the antibody by deriving the human framework sequence used for antibody reshaping from the human variable region that is most homologous or similar to the non-human-, particularly the rodent-derived variable region. This will also maximise the likelihood that affinity will be retained in the reshaped antibody.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6796

[0321] At its simplest level, the "best fit" strategy involves comparing the donor rodent V-region with all known human V-region amino acid sequences, and then selecting the most homologous to provide the acceptor framework regions for the humanization exercises. In reality there are several other factors which should be considered, and which may influence the final selection of acceptor framework regions. Molecular modelling predictions may be used in this regard prior to any experimental work in an attempt to maximise the affinity of the resultant reshaped antibody. Essentially, the goal of the modelling is to predict which key residues (if any) of the most homologous human framework should be left as in the rodent to obtain the best affinity in the reshaped antibody.

[0322] In one embodiment of the invention, the CDRs are obtainable from mouse monoclonal antibody, particularly from mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" throughout the application) described in co-pending application EP 05 02 7092.5 filed 12.12.2005, the disclosure of which is incorporated herein by reference.

[0323] Hybridoma cells FP-12H3-C2, producing mouse monoclonal antibody ACI-01-Ab7C2 (named "mC2" and hC2 for the humanized C2 antibody, throughout the application) were deposited 01 December 2005 in co-pending application no EP05027092.5 with the "Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in Braunschweig, Mascheroder Weg 1 B, 38124 Braunschweig, under the provisions of the Budapest Treaty and given accession no DSM ACC2750.

[0324] The mouse antibody may be raised against a supramolecular antigenic construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide, particularly of β-amyloid peptide $A\beta_{1\text{-}15}$, $A\beta_{1\text{-}16}$ and $A\beta_{1\text{-}16(\Delta 14)}$, modified with a hydrophobic moiety such as, for example, palmitic acid or a hydrophilic moiety such as, for example, polyethylene glycol (PEG) or a combination of both, wherein the hydrophobic and hydrophilic moiety, respectively, is covalently bound to each of the termini of the antigenic peptide through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophobic and hydrophilic moiety to the peptide fragment. When a PEG is used as the hydrophilic moiety, the free PEG termini is covalently bound to phosphatidylethanolamine or any other compound suitable to function as

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6797

the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome.

[0325] In particular, a mouse antibody may be raised against a supramolecular antigenic construct comprising an antigenic peptide corresponding to the amino acid sequence of the β-amyloid peptide $A\beta_{1-16}$ modified with a hydrophilic moiety such as, for example, polyethylene glycol (PEG) hydrophilic moiety is covalently bound to each of the termini of the antigenic peptide through at least one, particularly one or two amino acids such as, for example, lysine, glutamic acid and cysteine or any other suitable amino acid or amino acid analogue capable of serving as a connecting device for coupling the hydrophobic and hydrophilic moiety to the peptide fragment. When a PEG is used as the hydrophilic moiety, the free PEG termini are covalently bound to phosphatidylethanolamine or any other compound suitable to function as the anchoring element, for example, to embed the antigenic construct in the bilayer of a liposome.

[0326] In an embodiment of the invention, a chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is provided which comprises in the variable region at least one CDR of non-human origin embedded in one or more human- or primate-derived framework regions and combined with a constant region derived from a human or primate source antibody, which chimeric antibody or a fragment thereof, or a humanized antibody or a fragment thereof is capable of specifically recognizing and binding β-amyloid monomeric peptide.

[0327] The CDRs contain the residues most likely to bind antigen and must be retained in the reshaped antibody. CDRs are defined by sequence according to Kabat *et al.*, Sequence of Proteins of Immunological Interest, 5th Edition, The United States Department of Health and Human Services, The United States Government Printing Office, 1991. CDRs fall into canonical classes (Chothia *et al*, 1989 *Nature*, 342, 877-883) where key residues determine to a large extent the structural conformation of the CDR loop. These residues are almost always retained in the reshaped antibody.

[0328] In the process for preparing a humanized antibody according to the invention, the amino acid sequences of the C2 heavy chain and light chain variable regions (V_H and V_K) are compared to rodent antibody V_H and V_K sequences in the NCBI and Kabat databases.

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6798

[0329] The closest match mouse germ line gene to C2 V_K is bb1, Locus MMU231201, (Schable *et al*, 1999). A comparison reveals that two amino acids differ from this germ line sequence, both located within CDRL1. Mature murine antibodies with similar, but not identical, sequence can be found. Several have an identical CDRL2 and identical CDRL3, but the CDRL1 of C2 seems to be unique. Comparison with human germ line V_K sequences shows that genes from subgroup V_KII are the best match for C2 V_K (Cox *et al*, 1994). C2 V_K can thus be assigned to Kabat subgroup MuV_KII.Sequence.

[0330] DPK15 together with the human J region HuJ_K1 may be selected to provide the acceptor framework sequences for the humanized V_K.

[0331] The residues at the interface between the variable light and heavy chains have been defined (Chothia *et al*, 1985 *J. Mol. Biol.*, 186, 651-663). These are usually retained in the reshaped antibody. The Phe at position 87 of mouse C2 V_K is unusual at the interface, where a Tyr is more common in the V_KII subgroup, indicating that this framework residue may be important for antibody activity. Tyr 87 is present in the human germline and humanized C2VK.

[0332] The humanized V_K sequences thus may be designed such that the C2HuVK1 consists of mouse C2 V_K CDRs with frameworks from DPK 15 and human J_K1. In a specific embodiment of the invention, murine residues may be substituted in the human framework region at positions 45, and/or 87, and/or 50 and/or 53. Residue 45 may be involved in supporting the conformation of the CDRs. Residue 87 is located at the interface of the V_H and V_K domains. Therefore these residues may be critical for maintenance of antibody binding.

[0333] The closest match mouse germ line gene to C2 V_H AF is VH7183, Locus AF120466, (Langdon *et al*, 2000). Comparison with human germ line V_H sequences shows that genes from subgroup V_HIII are the best match for C2 V_H. C2 V_H AF can be assigned to Kabat subgroup MuV_HIIID. Sequence DP54 together with the human J region HuJ_H6 can be selected to provide the acceptor framework sequences for the humanized V_H.

[0334] The comparison shows that there are nine amino acid differences between the C2 VH sequences and the human acceptor germ line sequence DP54 and J_H6, most being located within CDRH2. Mature murine antibodies with identical or similar (one residue different) CDRH1 or with similar CDRH2 (one residue different) are found, but none with all three

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6799

CDRs identical to C2 V_H AF. CDRH3 of C2 antibody is unusually short, consisting of only three residues. However, other antibodies are found in the database with CDRH3 of this length. Residue 47 of C2 V_H is Leu rather than the more common Trp, and residue 94 is Ser rather than the normal Arg, indicating that these framework residues may be important for antibody activity.

[0335] Various humanized V_H sequences may be designed. C2HuVH1 consists of C2 V_H AF CDRs with frameworks from DP54 and HuJ_H6. In a specific embodiment of the invention, murine residues may be substituted in the human framework region at positions 47 or 94 or both. Residue 47 in framework 2 makes contact both with the CDRs and with the V_K domain. Residue 94 may be involved in supporting the conformation of the CDRs. Therefore these residues may be critical for maintenance of antibody binding.

[0336] Different HCVR and LCVR regions may be designed which comprise the non-human CDRs obtainable from the donor antibody, for example, a murine antibody, embedded into the native or modified human- or primate-derived framework regions. The modification may particularly concern an exchange of one or more amino acid residues within the framework region by non-human residues, particularly murine residues, more commonly found in this position in the respective subgroups or by residues which have similar properties to the ones more commonly found in this position in the respective subgroups.

[0337] The modification of the framework region the framework sequences serve to hold the CDRs in their correct spatial orientation for interaction with antigen, and that framework residues can sometimes even participate in antigen binding. In one embodiment of the invention measures are taken to further adapt the selected human framework sequences to make them most similar to the sequences of the rodent frameworks in order to maximise the likelihood that affinity will be retained in the reshaped antibody.

[0338] Accordingly, murine residues in the human framework region may be substituted. In particular, murine residues may be substituted in the human framework region of the Heavy Chain Variable (HCVR) region at positions 47 or 94 or both and in the human framework region of the Light Chain Variable (LCVR) region at positions 45 and/or 87 and/or 50 and/or 53, respectively.

[0339] The residues found in the above indicated positions in the human framework region may be exchanged by murine residues more commonly found in this position in the

Fujirebio Diagnostics, Inc. v. Quanterix Corp.
EX-1045
6800