Antibody	Reference / Source
9F1	santa cruz biotechnology, inc.
19H11	santa cruz biotechnology, inc.
1B11F3	santa cruz biotechnology, inc.
310-03	santa cruz biotechnology, inc.
79010Y	santa cruz biotechnology, inc.
16E9	santa cruz biotechnology, inc.
19B8	santa cruz biotechnology, inc.
3G5	santa cruz biotechnology, inc.
Mcl	santa cruz biotechnology, inc.
9C4	santa cruz biotechnology, inc.
3H2	santa cruz biotechnology, inc.
6E10	santa cruz biotechnology, inc.
4H309	santa cruz biotechnology, inc.
3H530	santa cruz biotechnology, inc.

[00113] In a specific embodiment, the CDRs of a safe and functional antibody of the invention are as follows: CDR1 of the heavy chain has the amino acid sequence of SEQ ID NO:1; CDR2 of the heavy chain has the amino acid sequence of SEQ ID NO:2; CDR3 of the heavy chain has the amino acid sequence of SEQ ID NO:3; CDR1 of the light chain has the amino acid sequence of SEQ ID NO:4; CDR2 of the light chain has the amino acid sequence of SEQ ID NO:5; and CDR3 of the light chain has the amino acid sequence of SEQ ID NO:6.

[00114] In another specific embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:7. In a more specific embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:7.

[00115] In another specific embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:10. In a more specific embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:10.

[00116] In an even more specific embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:7 and the heavy chain variable region of the safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:10.

[00117] In one embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at 100% identical to the amino acid sequence of SEQ ID NO:7 and the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:10.

[00118] The CDRL2 sequence ("KVSNRFS" (SEQ ID NO:5)) of the mMABT antibody may be modified slightly without adversely affecting antibody activity. Conservative substitutions may be made through exchange of R for K at position 50 and S for N at position 53. The two alternative CDRL2 sequences are therefore "RVSNRFS" and "KVSSRFS", respectively. These are incorporated into the murine VK sequence with no other changes, as mMABT VK-R and mMABT VK-S, respectively.

5.3.3 Construction of Antibodies

[00119] In certain embodiments, the CDRs of an antibody that binds specifically to an amyloid protein or its pathological form, such as beta amyloid, are placed into an antibody with constant regions consistent with a safe and functional antibody of the invention (Section 5.3.1). In certain embodiments, the variable regions of an antibody that binds specifically to an amyloid protein or its pathological form, such as beta amyloid, are combined with constant regions consistent with a safe and functional antibody of the invention (Section 5.3.1).

[00120] Methods for constructing humanized antibodies are well-known in the art. For example, methods for generating humanized antibodies are described in International Patent Application No. PCT/US2007/073504 published as WO2008/011348, which is incorporated herein by reference in its entirety.

Claims

1. A method for testing the safety of an anti-beta amyloid antibody, wherein the method comprises

a. incubating microglia cells with beta amyloid in the presence of the anti-beta amyloid antibody; and

b. measuring p38 MAP kinase activation in microglia cells in the presence of the anti-beta amyloid antibody; and

c. identifying the anti-beta amyloid antibody as safe if the anti-beta amyloid antibody induces an intermediate level of p38 MAP kinase activation in the microglia cells,

wherein the intermediate level of p38 MAP kinase activation is a level higher than a level of activation of p38 MAP kinase in the presence of beta amyloid without the anti-beta amyloid antibody, but lower than a level of activation of p38 MAP kinase in the presence of beta amyloid and an IgG1 antibody that specifically binds beta amyloid, and wherein the IgG1 antibody comprises a human IgG1 constant region.

2. A method for monitoring the course of treatment of amyloidosis in a patient being treated with an anti-beta amyloid antibody, wherein the method comprises a. measuring the degree of p38 MAP kinase activation in microglia cells obtained from the patient; and b. adjusting the course of treatment if the degree of p38 MAP kinase activation is different from an intermediate level of p38 MAP kinase activation in the microglia cells, wherein the intermediate level of p38 MAP kinase activation is a level higher than a level of activation of p38 MAP kinase in the absence of the course of treatment, but lower than a level of activation of p38 MAP kinase in the presence of beta amyloid and an IgG1 antibody that specifically binds beta amyloid, and wherein the IgG1 antibody comprises a human IgG1 constant region.

3. The method of claim 1, further comprising the step of testing the neuroprotective activity of the anti-beta amyloid antibody by measuring uptake of beta amyloid in the microglia cells in the presence and absence of the anti-beta amyloid antibody, wherein an increase in uptake of beta amyloid peptide by microglia cells as compared to uptake of beta amyloid by microglia cells in the absence of the anti-beta amyloid antibody indicates that the anti-beta amyloid antibody has neuroprotective activity.

4. The method of claim 2, wherein the beta amyloid is Aβ1-42 oligomers.

5. The method of claim 1 or 3, wherein the beta amyloid is Aβ1-42 oligomers.

6. The method of claim 2 or 4, wherein the amyloidosis is Alzheimer's disease.

7. The method of any one of claims 1-5, wherein the anti-beta amyloid antibody is safe and functional for the treatment of an amyloidosis associated with beta-amyloid deposits.

8. The method of claim 7, wherein the amyloidosis is Alzheimer's Disease.

9. The method of claim 8, wherein no inflammatory side effects are observed following administration of the antibody to a patient with Alzheimer's disease.

10. The method of claim 8, wherein any inflammatory side effects observed are not so severe that treatment of a patient with Alzheimer's Disease with the anti-beta antibody has to be interrupted.

11. The method of claim 2, 4 or 6, wherein adjusting the course of treatment comprises adjusting an administration dosage and/or administration frequency of a treatment.

12. The method of claim 11, wherein the administration dosage and/or administration frequency is reduced if the degree of p38 MAP kinase activation is above the intermediate level.

13. The method of claim 11, wherein the administration dosage and/or administration frequency is increased if the p38 MAP kinase activation level is below the intermediate level.

14. The method of claim 2, 4, 6, 11, 12 or 13 further comprising co-administering a modulator of the p38 MAP kinase signaling pathway to adjust the degree of p38 MAP kinase activation to intermediate levels.

15. The method of claim 14, wherein the modulator of the p38 MAP kinase signaling pathway is an inhibitor of p38 MAP kinase signaling pathway if the degree of p38 MAP kinase activation is above the intermediate level.

16. The method of claim 14, wherein the modulator of the p38 MAP kinase signaling pathway is an activator of p38 MAP kinase signaling pathway if the degree of p38 MAP kinase activation is below the intermediate level.

17. The method of any one of claims 1-16, wherein the activation of p38 MAP kinase in microglia cells is between 5%-15%; 10%-20%; 15%-25%; 20%-30%; 35%-45%; 40%-50%; or 45%-55% above p38 MAP kinase activation in microglia cells in the absence of the anti-beta amyloid antibody.

18. The method of any one of claims 1-17, wherein the anti-beta amyloid antibody comprises:

a. a light chain variable region complementarity determining region (CDR) 1, CDR2 and CDR3 comprising the amino acid sequence of the light chain variable region CDR1, CDR2 and CDR3, respectively of an anti-beta amyloid antibody listed in Table 2; and/or

b. a heavy chain variable region CDR1 having the amino acid sequence of the heavy chain variable region CDR1 of an anti-beta amyloid antibody listed in Table 2.

19. The method of claim 18, wherein the CDR1, CDR2, and CDR3 of the light chain variable region are all derived from the same anti-beta amyloid antibody listed in Table 2.

20. The method of claim 18, wherein the CDR1, CDR2, and CDR3 of the heavy chain variable region are all derived from the same anti-beta amyloid antibody listed in Table 2.

21. The method of claim 18, wherein the CDR1, CDR2, and CDR3 of the light chain variable region and the CDR1, CDR2, and CDR3 of the heavy chain variable region are all derived from the same anti-beta amyloid antibody listed in Table 2.

22. The method of any one of claims 1-21, wherein the anti-beta amyloid antibody has the effector region of an IgG4 antibody.

23. The method of any one of claims 1-21, wherein the anti-beta amyloid antibody is a non-IgG1 antibody which does not comprise a human IgG1 constant region.

24. The method of any one of claims 1-21, wherein the IgG1 antibody is the same anti-beta amyloid antibody having a human IgG1 isotype.

25. The method of any one of claims 1-21, wherein the anti-beta amyloid antibody has a human IgG4 isotype and the IgG1 antibody is the same anti-beta antibody with a human IgG1 isotype.

26. The method of any one of claims 1-25, wherein the IgG1 antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

Antibody	Reference / Source
9F1	santa cruz biotechnology, inc.
19H11	santa cruz biotechnology, inc.
1B11F3	santa cruz biotechnology, inc.
310-03	santa cruz biotechnology, inc.
79010Y	santa cruz biotechnology, inc.
16E9	santa cruz biotechnology, inc.
19B8	santa cruz biotechnology, inc.
3G5	santa cruz biotechnology, inc.
Mcl	santa cruz biotechnology, inc.
9C4	santa cruz biotechnology, inc.
3H2	santa cruz biotechnology, inc.
6E10	santa cruz biotechnology, inc.
4H309	santa cruz biotechnology, inc.
3H530	santa cruz biotechnology, inc.

[00113] In a specific embodiment, the CDRs of a safe and functional antibody of the invention are as follows: CDR1 of the heavy chain has the amino acid sequence of SEQ ID NO:1; CDR2 of the heavy chain has the amino acid sequence of SEQ ID NO:2; CDR3 of the heavy chain has the amino acid sequence of SEQ ID NO:3; CDR1 of the light chain has the amino acid sequence of SEQ ID NO:4; CDR2 of the light chain has the amino acid sequence of SEQ ID NO:5; and CDR3 of the light chain has the amino acid sequence of SEQ ID NO:6.

[00114] In another specific embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:7. In a more specific embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:7.

[00115] In another specific embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:10. In a more specific embodiment, the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:10.

[00116] In an even more specific embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:7 and the light chain variable region of the safe and functional antibody of the invention has an amino acid sequence that is at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or at least 99% identical to the amino acid sequence of SEQ ID NO:10.

[00117] In one embodiment, the heavy chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is at 100% identical to the amino acid sequence of SEQ ID NO:7 and the light chain variable region of a safe and functional antibody of the invention has an amino acid sequence that is 100% identical to the amino acid sequence of SEQ ID NO:10.

[00118] The CDRL2 sequence ("KVSNRFS" (SEQ ID NO:5)) of the mMABT antibody may be modified slightly without adversely affecting antibody activity. Conservative substitutions may be made through exchange of R for K at position 50 and S for N at position 53. The two alternative CDRL2 sequences are therefore "RVSNRFS" and "KVSSRFS", respectively. These are incorporated into the murine VK sequence with no other changes, as mMABT VK-R and mMABT VK-S, respectively.

5.3.3 Construction of Antibodies

[00119] In certain embodiments, the CDRs of an antibody that binds specifically to an amyloid protein or its pathological form, such as beta amyloid, are placed into an antibody with constant regions consistent with a safe and functional antibody of the invention (Section 5.3.1). In certain embodiments, the variable regions of an antibody that binds specifically to an amyloid protein or its pathological form, such as beta amyloid, are combined with constant regions consistent with a safe and functional antibody of the invention (Section 5.3.1).

[00120] Methods for constructing humanized antibodies are well-known in the art. For example, methods for generating humanized antibodies are described in International Patent Application No. PCT/US2007/073504 published as WO2008/011348, which is incorporated herein by reference in its entirety.

What is claimed is:

1. A method for testing the safety of an agent, wherein the method comprises
   a. incubating microglia cells with neurotoxic Aβ1-42 oligomers and the agent; and
   b. measuring p38 MAP kinase activation in microglia cells.

2. A method for monitoring the course of treatment of amyloidosis, such as Alzheimer's Disease, in a patient, wherein the method comprises measuring the degree of p38 MAP kinase activation in microglia cells obtained from the patient.

3. A method for testing the neuroprotective activity of an agent, wherein the method comprises
   a. incubating microglia cells with neurotoxic Aβ1-42 oligomers and agent; and
   b. measuring uptake of beta amyloid peptide by microglia cells.

4. The method of claim 2, wherein the amyloidosis is treated with an anti beta-amyloid antibody.

5. The method of claim 1, further comprising the step of testing the neuroprotective activity of the agent by measuring uptake of beta amyloid by the microglia cells in the presence and absence of the agent.

6. The method of claim 3, further comprising measuring p38 MAP kinase activation in microglia cells.

7. The method of claim 1, 3, 5, or 6, wherein the agent is an anti-beta amyloid antibody.

8. Use of a humanized non-IgG1 anti-beta amyloid antibody in the manufacture of a medicament for treating amyloidosis, such as Alzheimer's Disease, wherein the antibody is to be administered to a patient in need thereof in a safe and functional amount or dosage regimen, wherein said safe and functional amount or dosage regimen results in activation of p38 MAP kinase in microglia cells of the patient at a level above the level of activation of p38 MAP kinase in the absence of the humanized non-IgG1 anti-beta amyloid antibody but below the level of p38 MAP kinase activation in the presence of the same concentration of an IgG1 anti-beta amyloid antibody which binds to beta amyloid with the same Kd as the humanized non-IgG1 anti-beta amyloid antibody.

9. The method of claim 1, 5, or 6, wherein the activation of p38 MAP kinase in microglia cells is between 5%-15%; 10%-20%; I5%-25%; 20%-30%; 35%-45%; 40%-50%; or 45%-55% above p38 MAP kinase activation in microglia cells in the absence of the agent.

10. The use of claim 8, wherein the activation of p38 MAP kinase in microglia cells of the patient is

~~between 5%-15%; 10%-20%; 15%-25%; 20%-30%; 35%-45%; 40%-50%; or 45%-55% above p38 MAP~~ kinase activation in microglia cells of the patient in the absence of the humanized non-IgG1 anti-beta amyloid antibody.

11. A method for producing a safe and functional humanized anti-beta-amyloid antibody or for improving the safety of a non-IgG4 anti-beta amyloid antibody comprising replacing the constant region in a non-IgG4 antibody having an amino acid sequence of the variable region that functionally binds beta-amyloid with a constant region derived from an IgG4 antibody or from a non-IgG1 antibody, and optionally the non-IgG4 antibody is an IgG1 antibody.

12. A method for testing the neuroprotective activity of an anti-beta amyloid antibody, which comprises

a. obtaining primary, mixed cortical cell culture;
b. incubating the cell culture with neurotoxic Aβ1-42 oligomers and the anti-beta amyloid antibody; and
c. measuring neuronal survival rate.

13. The method of claim 12, wherein the neuronal survival rate is measured by metabolic turnover as determined by mitochondrial oxidation of MTT or by ATP release.

14. The method of claim 4 or 7 wherein the anti-beta amyloid antibody comprises:

a. a light chain variable region CDR1, CDR2 and CDR3 comprising the amino acid sequence of the light chain variable region CDR1, CDR2 and CDR3, respectively of an anti-beta amyloid antibody listed in Table 2; and/or
b. a heavy chain variable region CDR1 having the amino acid sequence of the heavy chain variable region CDR1 of an anti-beta amyloid antibody listed in Table 2, optionally the CDR1, CDR2, and CDR3 of the light chain variable region and/or the CDR1, CDR2, and CDR3 of the heavy chain variable region are all derived from the same anti-beta amyloid antibody listed in Table 2.

15. The use of claim 8, wherein the humanized non-IgG1 anti-beta amyloid antibody comprises a light chain variable region comprising the amino acid sequence of SEQ ID NO: 7 and a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 10.

16. A humanized anti-beta-amyloid antibody obtainable by the method of claim 10, wherein the humanized anti-beta-amyloid antibody comprises i) an amino acid sequence of a variable region which binds beta-amyloid derived from a non-IgG4 humanized antibody and ii) an amino acid sequence of a constant region derived from an IgG4 antibody.

17. The humanized anti-beta-amyloid antibody of claim 16 wherein (i) the non-IgG4 humanized

~~antibody is selected from the group consisting of the anti-beta amyloid antibodies listed in Table 2, or (ii)~~ the amino acid sequence of the variable region which binds beta-amyloid is derived from an IgG1 humanized antibody and the amino acid sequence of a constant region is derived from an IgG4 antibody or from a non-IgG1 antibody.

18. A pharmaceutical composition comprising a safe and functional amount of the humanized antibody of claim 16 or 17 and a pharmaceutical acceptable carrier.

19. The antibody of claim 16 or 17 wherein the anti-beta amyloid antibody comprises:

a. a light chain variable region CDR1 having the amino acid sequence of the light chain variable region CDR1 of an anti-beta amyloid antibody listed in Table 2;

b. a light chain variable region CDR2 having the amino acid sequence of the light chain variable region CDR2 of an anti-beta amyloid antibody listed in Table 2; and

c. a light chain variable region CDR3 having the amino acid sequence of the light chain variable region CDR3 of an anti-beta amyloid antibody listed in Table 2.

20. The antibody of claim 16 or 17 wherein the anti-beta amyloid antibody comprises:

a. a heavy chain variable region CDR1 having the amino acid sequence of the heavy chain variable region CDR1 of an anti-beta amyloid antibody listed in Table 2;

b. a heavy chain variable region CDR2 having the amino acid sequence of the heavy chain variable region CDR2 of an anti-beta amyloid antibody listed in Table 2; and

c. a heavy chain variable region CDR3 having the amino acid sequence of the heavy chain variable region CDR3 of an anti-beta amyloid antibody listed in Table 2.

21. The method of claim 19 wherein the CDR1, CDR2, and CDR3 of the light chain variable region and the CDR1, CDR2, and CDR3 of the heavy chain variable region are all derived from the same anti-beta amyloid antibody listed in Table 2.