



US009908933B2

(12) **United States Patent**
Miles et al.

(10) **Patent No.:** **US 9,908,933 B2**
(45) **Date of Patent:** **Mar. 6, 2018**

(54) **ANTIBODY THERAPY FOR AMYLOID BETA DISEASE**

2317/565 (2013.01); C07K 2317/76 (2013.01);
G01N 2333/4709 (2013.01); G01N 2800/2821
(2013.01); G01N 2800/368 (2013.01)

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(58) **Field of Classification Search**
CPC C07K 16/18; C07K 2317/24; C07K
2317/33; C07K 2317/34; C07K 2317/55;
C07K 2317/565; C07K 2317/76
See application file for complete search history.

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(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

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(21) Appl. No.: **14/910,172**

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(22) PCT Filed: **Aug. 5, 2014**

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(86) PCT No.: **PCT/AU2014/050172**

§ 371 (c)(1),
(2) Date: **Feb. 4, 2016**

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(87) PCT Pub. No.: **WO2015/017900**

PCT Pub. Date: **Feb. 12, 2015**

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(65) **Prior Publication Data**

US 2016/0194385 A1 Jul. 7, 2016

Supplementary European Search report issued in corresponding European application No. EP14834839, dated Mar. 7, 2017.
International Search Report for corresponding application No. PCT/AU2014/050172 dated Nov. 27, 2014.

(30) **Foreign Application Priority Data**

Aug. 5, 2013 (AU) 2013902922

* cited by examiner

(51) **Int. Cl.**

C07K 16/18 (2006.01)
A61K 39/395 (2006.01)
G01N 33/53 (2006.01)
C07H 21/04 (2006.01)
G01N 33/68 (2006.01)

Primary Examiner — Kimberly A. Ballard

(52) **U.S. Cl.**

CPC **C07K 16/18** (2013.01); **G01N 33/689**
(2013.01); **G01N 33/6896** (2013.01); **C07K**
2299/00 (2013.01); **C07K 2317/24** (2013.01);
C07K 2317/33 (2013.01); **C07K 2317/34**
(2013.01); **C07K 2317/55** (2013.01); **C07K**

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(57) **ABSTRACT**

The present disclosure relates generally to a protocol to treat or prevent diseases or conditions associated with pathological forms of amyloid beta (Aβ), including Alzheimer’s disease. Enabled herein is a reagent for use in the treatment and prophylaxis of Aβ-associated pathological conditions.

16 Claims, 3 Drawing Sheets

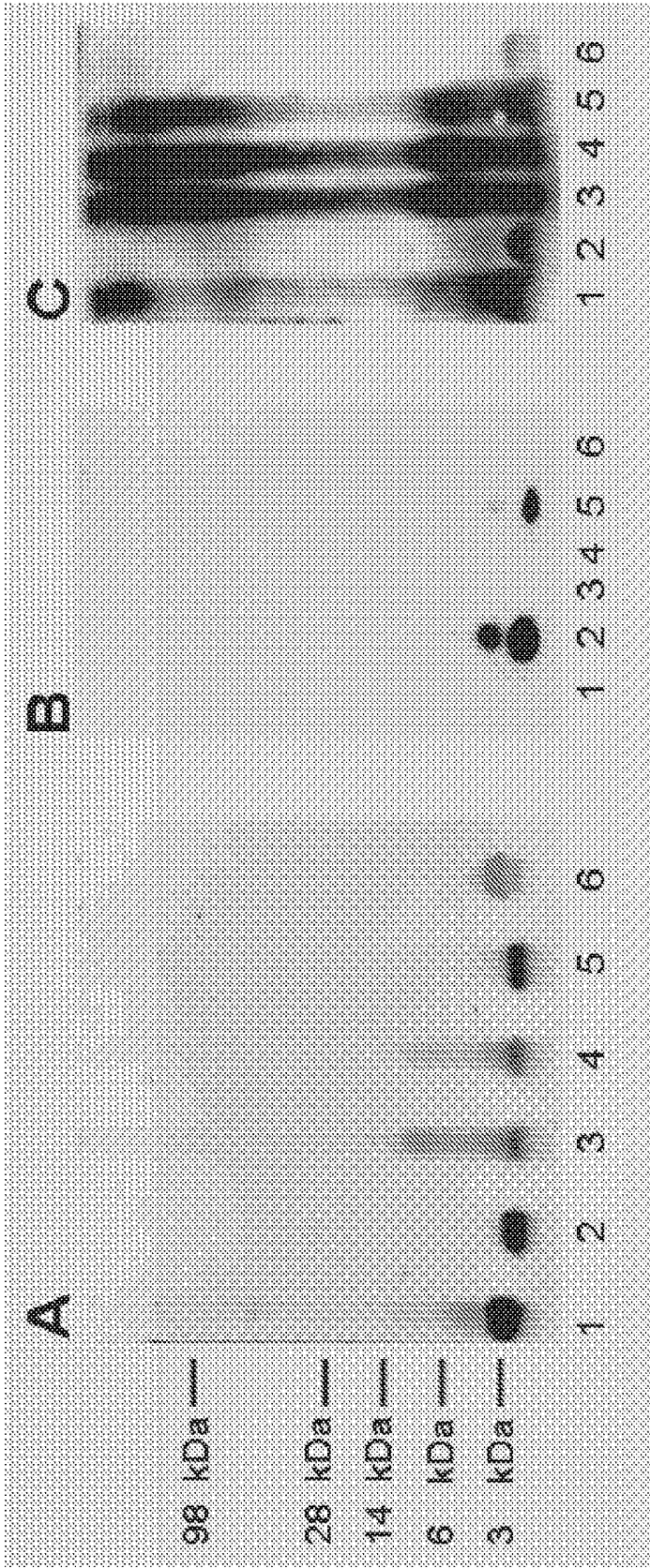


Figure 1

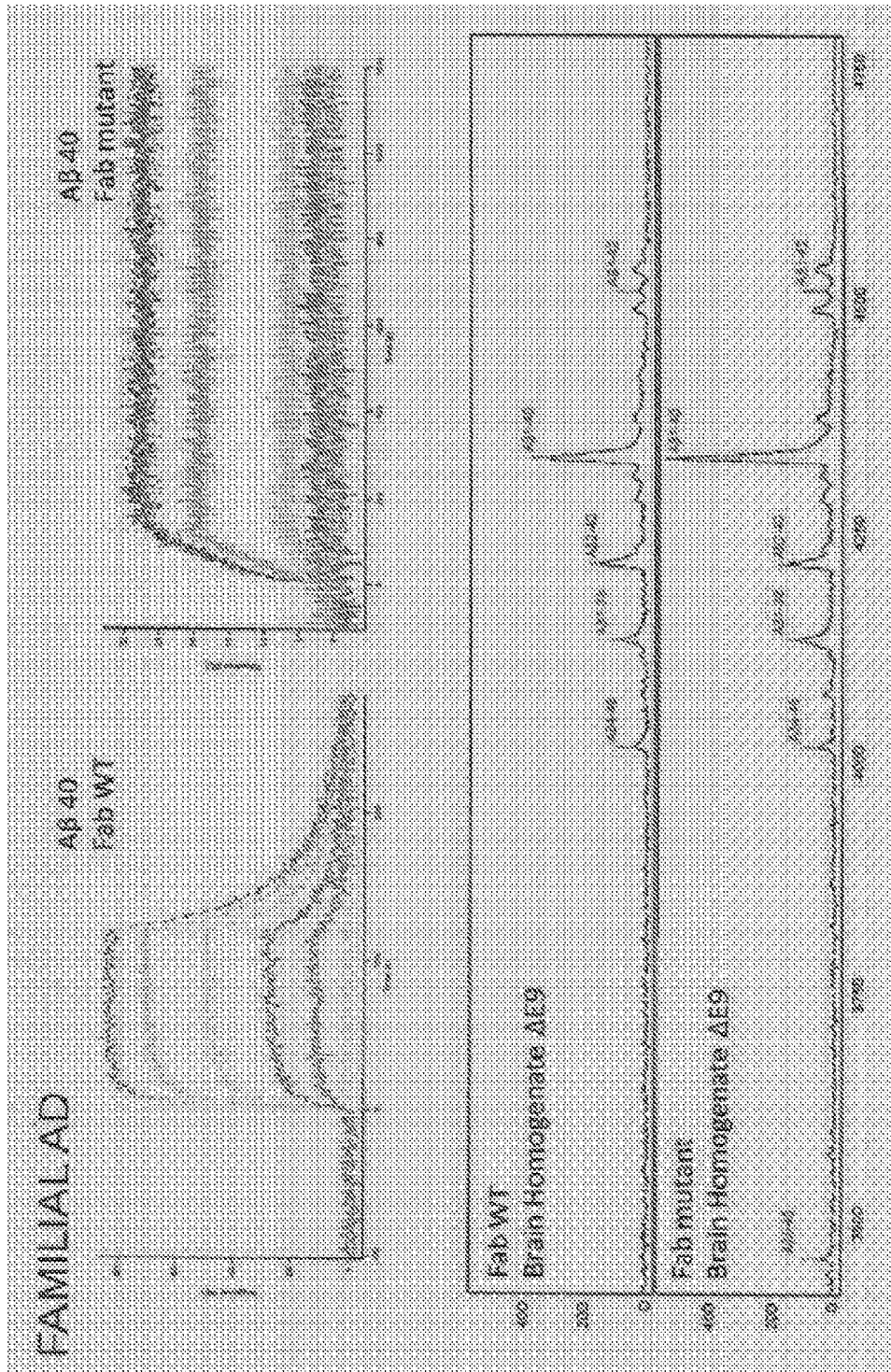


Figure 2

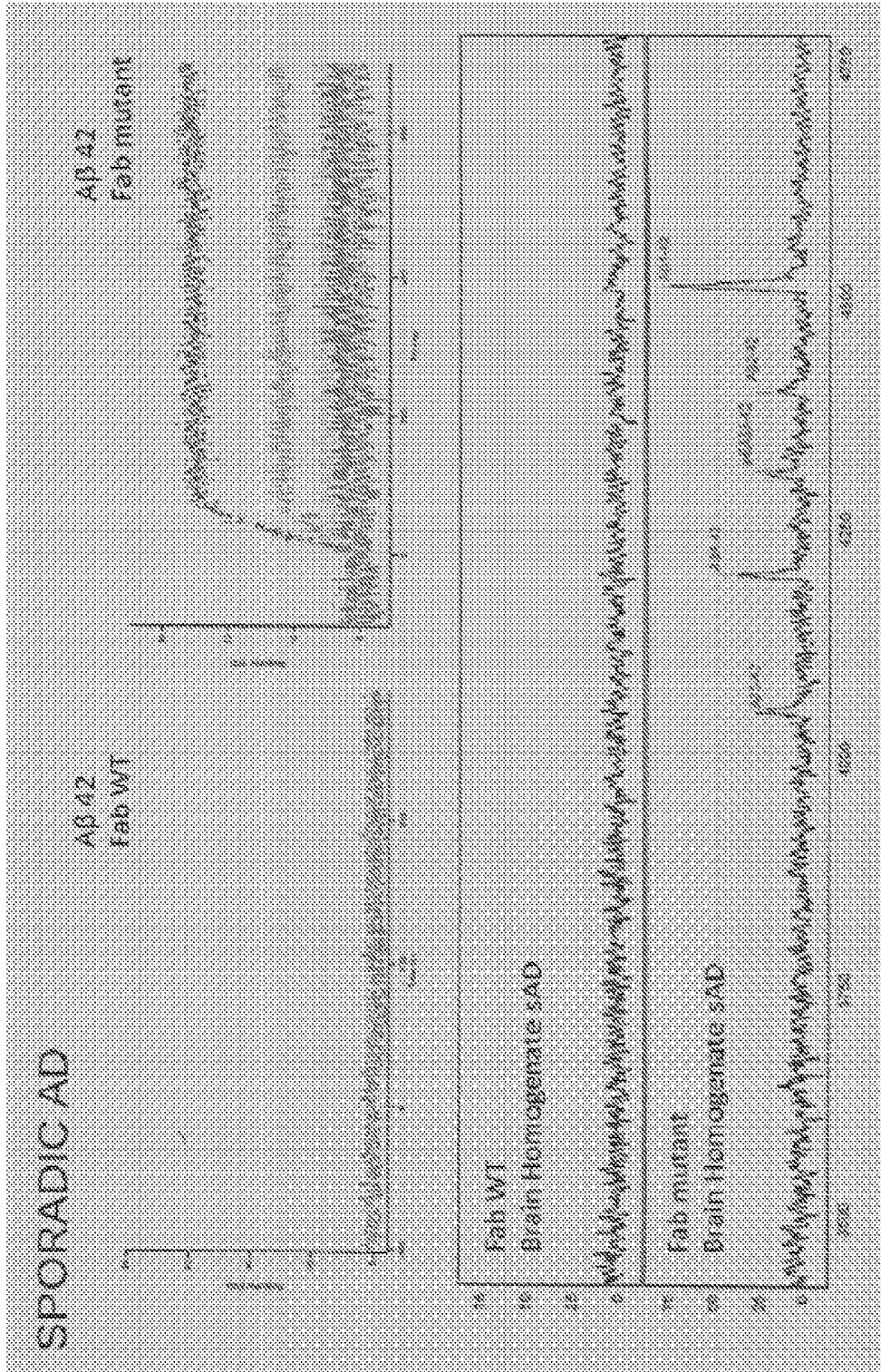


Figure 3

ANTIBODY THERAPY FOR AMYLOID BETA DISEASE

FILING DATA

This application is associated with and claims priority from Australian Provisional Patent Application No. 2013902922, filed on 5 Aug. 2013, entitled "An antibody therapy for amyloid beta disease", the entire contents of which, are incorporated herein by reference.

BACKGROUND

Field

The present disclosure relates generally to a protocol to treat or prevent diseases or conditions associated with pathological forms of amyloid beta ($A\beta$), including Alzheimer's disease. Enabled herein is a reagent for use in the treatment and prophylaxis and diagnosis of $A\beta$ -associated pathological conditions.

Prior Art

Bibliographic details of the publications referred to by author in this specification are collected alphabetically at the end of the description.

Reference to any prior art in this specification is not, and should not be taken as, an acknowledgment or any form of suggestion that this prior art forms part of the common general knowledge in any country.

Alzheimer's disease is a degenerative brain disorder characterized histologically by neuritic plaques found primarily in association with the cortex, limbic system and basal ganglia. These neuritic plaques comprise a cleavage product of amyloid precursor protein (APP), a type I transmembrane glycoprotein.

The cleavage product of APP is β -amyloid peptide or $A\beta$. Incorrect processing of APP can result in pathological forms of $A\beta$ (Tanzi et al. (1996) *Neurobiol Dis* 3:159-168; Hardy (1996) *Ann Med* 28:255-258; Schenk et al. (1999) *Nature* 400:173-177). These pathological forms include $A\beta_{1-42}$ and $A\beta_{1-43}$ which have been detected as predominant species in the neurite plaques.

Bard et al. (2000) *Nature Medicine* 6:916-919 showed that peripheral administration of antibodies directed against $A\beta$ can reduce plaque burden. Bard et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:2023-2028 subsequently showed that Fc-mediated phagocytosis by microglial cells or macrophages is associated with plaque clearance. Hence, antibody therapy has the potential to treat Alzheimer's disease.

This is supported by non-Fc-mediated mechanisms being found to be associated with $A\beta$ clearance in immunotherapy (Bacskaï et al. (2002) *J. Neurosci.* 22:7873-7878; Das et al. (2003) *J. Neurosci.* 23:8532-9538).

One attempt at immunotherapy was the humanized antibody, Bapineuzumab or Bapi, developed by Pfizer and Johnson & Johnson. Bapi targets neurotoxic $A\beta$ (Salloway et al. (2009) *Neurology* 73:2061-2070) at the extreme N-terminus in a helical conformation (Miles et al. (2013) *Sci Rep* 3:1-8) [doi:10.1038/srep01302]. Such $A\beta$ forms with the N-terminal truncations comprise approximately 60% of $A\beta$ deposits in the Alzheimer's diseased brains. Bapi was, however, shown to be toxic at higher doses.

Another antibody is Solanezumab (Eli Lilly). This antibody targets monomeric $A\beta$ in the mid-region of the peptide and was partially efficacious in mild cases of Alzheimer's disease (Hardy (2014) *N Engl J Med* 370(4):377-378).

However, it appears that Solanezumab may be ineffective at reversing the symptoms in the later stages of Alzheimer's disease (Panza et al. (2014) *Expert Opin Biol Ther*:1-12 PMID 24981190).

Crenezumab (Genentech Inc.) also targets the mid-region of $A\beta$. It appears to stimulate microglia to a level sufficient to clear $A\beta$ but without inducing an inflammatory response (Adolfsson et al. (2012) *J. Neuroscience* 32(28):9677-9689).

Ponezumab (Pfizer) targets the C-terminal end of $A\beta_{1-40}$ but is unable to bind to elongated forms such as $A\beta_{1-42}$ and $A\beta_{1-43}$. Ponezumab was not efficacious in reducing biomarkers of Alzheimer's disease or cognitive decline (Liu et al. (2014) *Mol Neurobiol*:PMID 24733588). Another monoclonal antibody which has the same binding specificity as Ponezumab is referred to as Mab 2286 (Rosenthal et al. USSN 2011/038861, 2004/0146512 and 2007/0160616). Mab 2286 also does not bind to $A\beta_{1-42}$ and $A\beta_{1-43}$.

There is a need to further develop an immunotherapeutic approach to the treatment of disease conditions associated with toxic $A\beta$ forms and their diagnosis.

SUMMARY

Nucleotide and amino acid sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A summary of the sequence identifiers is provided in Table 1. A sequence listing is provided after the claims.

The present disclosure teaches the generation of an antibody and derivatives thereof which target $A\beta_{1-40}$ and its C-terminally extended forms including $A\beta_{1-42}$ and $A\beta_{1-43}$. The inventors analyzed the molecular basis of antibody engagement with $A\beta$. The 3D structure of a Fab binding fragment of the variable domain of Mab 2286 (referred to herein as "Fab 2286") was determined to near atomic resolution. Fab 2286 is a chimera comprising the mouse variable region of Mab 2286 transplanted onto a human scaffold. Fab 2286 comprises V_H and V_L from Mab 2286 and C_H and C_L from a human template. This antibody exhibits similar specificity as Ponezumab to the C-terminus of $A\beta_{1-40}$ and does not react with $A\beta_{1-42}$ or $A\beta_{1-43}$ or other elongated forms such as $A\beta_{3-42}$, $A\beta_{4-42}$, $A\beta_{pyroGlu3-42}$ and $A\beta_{pyroGlu11-42}$. The analysis of chimeric Fab 2286 revealed a hydrophobic cavity in the Fab 2286 surface suitable for binding amyloid beta peptide. The amino acid sequences of Ponezumab and Fab 2286 show little similarity in the complementarity determining regions, but comparison of the 3D structures (Protein Data Bank entries 3U0T and 3U0W, respectively) revealed some potential spatial similarities for ligand engagement. Structural analysis of the Ponezumab- $A\beta$ complex revealed a buried $A\beta$ terminal carboxyl location that excluded cross reactivity with C-terminally elongated $A\beta$ species. A modification is prepared of the proposed $A\beta_{1-40}$ C-terminus binding site of Fab 2286 at the glutamic acid (E; Glu) at residue 50 in the heavy variable chain of Fab 2286 [Glu50]. The substitution to another amino acid (Glu50Xaa), wherein Xaa is not glutamic acid, substantially improves affinity for the $A\beta_{1-40}$ ligand and enables binding to the C-terminally elongated $A\beta$ species. In an embodiment, Xaa is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, Xaa is a

basic amino acid such as arginine, lysine or histidine. In an embodiment, Xaa is alanine. In a particular embodiment, Xaa is arginine.

Accordingly, enabled herein is an antibody that specifically binds to the C-terminal end portion of A β ₁₋₄₀ and to C-terminally extended toxic forms thereof. In an embodiment, the antibody is a derivative of Fab 2286 and is referred to herein as a "Fab 2286-like antibody". Fab 2286 comprises the antigen binding fragment of Mab 2286 grafted onto a human scaffold constant region. In an embodiment, the instant disclosure teaches a Fab 2286-like antibody comprising an amino acid substitution at glutamic acid (E) in its heavy chain in the amino acid sequence WIGX to generate the sequence WIGX. In an embodiment, the substitution is to an amino acid selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the substitution is to a basic amino acid such as arginine, lysine or histidine. In an embodiment, the substitution is to an alanine. In an embodiment, the substitution is to an arginine. The Fab 2286-like antibody may have one or more other mutations. The Fab 2286-like antibody may be conjugated to any light and heavy chain constant region and be subject to mammalianization such as humanization (or deimmunization). In terms of testing the antibody in an animal model, the antibody may, for example, be subject to murinization.

Further enabled herein is a method for treating a disease or condition characterized by the presence of aberrant or toxic forms of A β including C-terminally elongated forms (e.g. A β ₁₋₄₂, A β ₁₋₄₃, A β ₃₋₄₂, A β ₄₋₄₂, A β _{pyroGlu3-42} and A β _{pyroGlu11-42}) as well as covalently cross-linked pathogenic forms of A β multimers. The method comprises the administration of a Fab 2286-like antibody comprising a mutation in its heavy chain variable region which enables it to bind to C-terminally elongated pathogenic forms of A β . The mutation is a Glu50Xaa substitution in SEQ ID NO: 1. An example is a Glu50Arg substitution as depicted in SEQ ID NO:2. Other modified forms of the heavy chain which includes the Glu50Arg substitution are provided in SEQ ID NOs:6 and 8. As indicated above, however, the glutamic acid is changed to an amino acid selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the amino acid is a basic amino acid such

as arginine, lysine or histidine. In an embodiment, the amino acid is alanine. In an embodiment, the amino acid is arginine.

Diseases and conditions contemplated herein include Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]). The subject antibody may also be used to capture and/or detect toxic forms of A β associated with the above-listed conditions. In terms of diagnosis, another condition contemplated herein is pre-eclampsia. Whilst pre-eclampsia and a neurological condition such as Alzheimer's disease share no clinical features in common, the pre-eclampsia susceptibility gene, STOX-1, is abundantly expressed in the brain and transactivates LRRTM3 in neural cells which promotes elevated A β processing. Hence, the presence of toxic forms of A β in urine is potentially indicative of pre-eclampsia. The Fab 2286-like antibody may act to promote microglial-mediated removal of pathogenic A β species in the brain and/or through the "peripheral sink" route to induce an equilibrium shift to remove A β from the brain to the blood stream where it is removed by various immune mechanisms.

The treatment may also include ameliorating symptoms or delaying onset of symptoms of an A β pathology such as Alzheimer's disease.

Another aspect contemplated herein is a method of detecting a toxic form of A β in a sample from a subject, the method comprising identifying binding between the A β form and Fab 2286-like antibody. The antibody may also be used to capture an A β form which is then detected by, for example, another antibody specific for an epitope on A β or by an anti-immunoglobulin antibody which binds to the Fab 2286-like antibody.

Diagnostic applications extend to Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]) and pre-eclampsia. Whilst a blood-based test is proposed for most conditions, for pre-eclampsia, a urine-based test is proposed. Cerebral spinal fluid may also be tested. In an embodiment, taught herein is a dipstick comprising Fab 2286-like antibody immobilized thereon for use in detecting toxic forms of A β in a biological sample. In an embodiment, the sample is urine and the condition diagnosed is pre-eclampsia.

TABLE 1

Summary of sequence identifiers	
SEQUENCE ID NO:	DESCRIPTION
1	Amino acid sequence of heavy chain variable region of Fab 2286 antibody
2	Amino acid sequence of heavy chain of Fab 2286-like antibody comprising Glu50Arg substitution
3	Amino acid sequence of light chain (hkappa) of Fab 2286 (and Fab 2286-like antibody)
4	Amino acid sequence of A β ₁₋₂₈
5	Amino acid sequence of A β ₁₋₄₀
6	Amino acid sequence of A β ₁₋₄₂
7	Amino acid sequence of A β ₁₋₄₃
8	Amino acid sequence of A β ₃₅₋₄₀

TABLE 1-continued

Summary of sequence identifiers	
SEQUENCE ID NO:	DESCRIPTION
9	Amino acid sequence of murinized Fab 2286-like antibody heavy chain conjugated to human gamma-1 immunoglobulin (hG1) heavy constant region with Glu50Arg substitution
10	Amino acid sequence of chimeric Fab 2286 antibody heavy chain variable region
11	Amino acid sequence of chimeric Fab 2286-like antibody heavy chain variable region
12	Nucleotide sequence of Mab 2286 heavy chain (variable domain and constant domain 1 [CH1])
13	Amino acid sequence of Mab 2286 heavy chain (variable domain and constant domain 1 [CH1])
14	Nucleotide sequence of Mab 2286 light chain
15	Amino acid sequence of Mab 2286 light chain
16	Nucleotide sequence of synthetic DNA construct encoding Fc portion of murine gamma heavy chain ((GenBank: AAA75163.1) of Mab 2286
17	Amino acid sequence of Fc portion of murine gamma heavy chain (GenBank: AAA75163.1) of Mab 2286
18	Nucleotide sequence of murine heavy B encoding Glu to Arg substitution
19	Amino acid sequence of murine heavy B with Glu to Arg substitution
20	Nucleotide sequence of murine light chain of Fab 2286-like antibody
21	Amino acid sequence of murine light chain of Fab 2286-like antibody

2. Amino acid abbreviations used herein are defined in Table 2.

TABLE 2

Amino acid three and single letter abbreviations		
Amino Acid	Three-letter Abbreviation	One-letter Symbol
Alanine	Ala	A
Arginine	Arg	R
Asparagine	Asn	N
Aspartic acid	Asp	D
Cysteine	Cys	C
Glutamine	Gln	Q
Glutamic acid	Glu	E
Glycine	Gly	G
Histidine	His	H
Isoleucine	Ile	I
Leucine	Leu	L
Lysine	Lys	K
Methionine	Met	M
Phenylalanine	Phe	F
Proline	Pro	P
Serine	Ser	S
Threonine	Thr	T
Tryptophan	Trp	W
Tyrosine	Tyr	Y
Valine	Val	V
Pyrrolysine	Pyl	O
Selenocysteine	Sec	U
Any residue	Xaa	X

BRIEF DESCRIPTION OF THE FIGURES

Some figures contain color representations or entities. Color photographs are available from the Patentee upon request or from an appropriate Patent Office. A fee may be imposed if obtained from a Patent Office.

FIG. 1A through C are photographic representations of SDS-PAGE analysis of synthetic A β preparations. Detected by (A) Coomassie stain, (B) anti-A β 40 C-terminus specific Mab 2286 Western Blot and (C) anti-A β 2-7 specific Mab WO2 Western Blot. In each panel, lanes represent: (1) A β ₁₋₄₀ aged for 21 days at room temperature in PBS; (2)

25 freshly prepared, from 2,2,2-trifluoroethanol (TFE)-treated and lyophilized stock, A β ₁₋₄₀; (3) A β ₁₋₄₂ aged for 21 days at room temperature in phosphile buffered saline (PBS); (4) freshly prepared, from TFE-treated and lyophilized stock, 30 A β ₁₋₄₂; (5) 1:1 mixture of A β ₁₋₄₀ and A β ₁₋₄₂ aged overnight at room temperature in PBS; (6) A β ₁₋₂₈ freshly prepared, from TFE-treated and lyophilized stock.

FIG. 2 is a graphical representation of the binding characteristics of Fab 2286 (A β Fab wt) and the Fab 2286-like 35 antibody (A β Fab mutant) in tissue from a familial Alzheimer's diseased brain and against synthetic peptide A β ₁₋₄₂.

FIG. 3 is a graphical representation of the binding characteristics of Fab 2286 (A β Fab wt) and the Fab 2286-like 40 antibody (A β Fab mutant) in tissue from a sporadic Alzheimer's diseased brain and against synthetic peptide A β ₁₋₄₂.

DETAILED DESCRIPTION

45 Throughout this specification, unless the context requires otherwise, the word "comprise" or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or method step or group of elements or integers or method steps but not the 50 exclusion of any element or integer or method step or group of elements or integers or method steps.

As used in the subject specification, the singular forms "a", "an" and "the" include plural aspects unless the context clearly dictates otherwise. Thus, for example, reference to "a mutation" includes a single mutation, as well as two or more mutations; reference to "an antibody" includes a single antibody, as well as two or more antibodies; reference to "the disclosure" includes a single reference to "the disclosure" and includes single and multiple aspects taught by the disclosure; and so forth. Aspects taught and enabled herein are encompassed by the term "invention". All such aspects are enabled within the width of the present invention.

Disclosed herein is an antibody which binds to A β ₁₋₄₀ ("A β ₄₀") as well as C-terminally elongated pathogenic forms of A β , including A β ₁₋₄₂ ("A β ₄₂") and A β ₁₋₄₃ ("A β ₄₃") as well as covalently linked multimers thereof. A "multimer" includes an oligomer. The antibody comprises a mature

heavy chain having the amino acid sequence as set forth in SEQ ID NO: 1 with the proviso that in the sequence WIGE, the glutamic acid (E) is substituted for another amino acid residue, i.e. Glu50Xaa. In an embodiment, Xaa is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, Xaa is a basic amino acid such as arginine, lysine or histidine. In an embodiment, Xaa is alanine. In an embodiment, Xaa is arginine such as depicted in SEQ ID NO:2. Other forms are provided in SEQ ID NOs:9 and 11. The amino acid sequence of its light chain is set forth in SEQ ID NO:3. The mature heavy chain is derived from an antigen-binding fragment of Mab 2286 (a Fab 2286 fragment) and the antibody of the present disclosure is referred to as an "Fab 2286-like antibody". It is also referred to as "A β Fab mutant". The Mab 2286 precursor antigen binding fragment is referred to as "Fab 2286" or "A β Fab wt". The Fab 2286-like antibody differs from Ponezumab and Mab 2286 by its ability to bind to C-terminally elongated A β species. These include A β ₁₋₄₂ and A β ₁₋₄₃ as well as A β ₃₋₄₂, A β ₄₋₄₂, A β _{pyroGlu3-42} and A β _{pyroGlu11-42}. The Fab 2286-like antibody may also comprise one or more additional amino acid substitutions, additions and/or deletions. In addition, the Fab 2286-like antibody may be conjugated to any heavy and light constant regions. For example, in an embodiment, the Fab 2286-like antibody is conjugated to human kappa-1 immunoglobulin (hkappa) light chain and human gamma-1 immunoglobulin (hG1) heavy chain constant regions. In one example, the heavy chain variable and constant regions (V_H+C_H) have an amino acid sequence set forth in SEQ ID NO:9. Furthermore, the antibody may be mammalianized so that it can be administered to a particular mammal such as a mouse, rat, pig, sheep or monkey. In an embodiment, the mammal is a human and the antibody is humanized or deimmunized. In terms of testing the antibody in an animal model such as a mouse model, it may also be subject to murinization.

Accordingly, enabled herein is an isolated Fab 2286-like antibody which binds to A β ₁₋₄₀ and a C-terminally elongated form thereof, the antibody comprising a modified Fab 2286 wherein a mature heavy chain variable region comprises an amino acid sequence as set forth in SEQ ID NO:1 with the proviso that the amino acid residue at position 50 is not glutamic acid, wherein the numbering of the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) represents residues 47 to 50 or comprising one or more other amino acid substitutions, additions and/or deletions to the amino acid sequence of SEQ ID NO:1.

Enabled herein is an isolated antibody comprising a mature heavy chain as set forth in SEQ ID NO:2 or one or more amino acid substitutions, additions and/or deletions to the amino acid sequence set forth in SEQ ID NO:2 provided that the amino acid residue at position 50 is arginine (R) [50 Arg]. In an embodiment, the amino acid sequence comprises at least 90% similarity to SEQ ID NO:2 provided it still contains the Glu50Arg substitution.

Enabled herein is an isolated antibody comprising a mature heavy chain as set forth in SEQ ID NO:9 or one or more amino acid substitutions, additions and/or deletions to the amino acid sequence set forth in SEQ ID NO:9 provided that the amino acid residue at position 50 is arginine (R) [50 Arg]. In an embodiment, the amino acid sequence comprises at least 90% similarity to SEQ ID NO:9 provided it still contains the Glu50Arg substitution.

Enabled herein is an isolated antibody comprising a mature heavy chain as set forth in SEQ ID NO:1 or one or more amino acid substitutions, additions and/or deletions to the amino acid sequence set forth in SEQ ID NO:11 provided that the amino acid residue at position 50 is arginine (R) [50 Arg]. In an embodiment, the amino acid sequence comprises at least 90% similarity to SEQ ID NO:1 provided it contains the Glu50Arg substitution.

To take into account variations in the heavy chain, in the amino acid sequence WIGE of SEQ ID NO:1 (mature heavy chain variable region of Fab 2286), the E is at position 50 and in the Fab 2286-like antibody, the sequence is WIGR (see SEQ ID NO:2, 9 and 11). This is also referred to herein as a Glu50Arg substitution of the mature heavy chain variable region of Fab 2286. However, the present invention extends to a substitution of E at position 50 to any amino acid residue such as alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine or selenocysteine. In an embodiment, the substitution is to a basic amino acid residue such as arginine, lysine or histidine. In an embodiment, the substitution is to an alanine. In an embodiment, the substitution is to an arginine. In an embodiment, the present invention extends to a Fab 2286-like antibody comprising a heavy chain variable region as set forth in SEQ ID NO: 1 with the exception that in the sequence WIGE at amino acids 47 to 50, the sequence is selected from WIGA, WIGR, WIGN, WIGD, WIGC, WIGQ, WJGG, WIGH, WIGI, WIGL, WIGK, WIGM, WIGF, WIGP, WIGS, WIGT, WIGW, WIGY, WIGV, WIGO and WIGU. In an embodiment, the sequence is WIGR, WIGK, WIGH, WIGA or WIGM. In an embodiment, the sequence is WIGR. Reference to "at least 90%" amino acid sequence similarity means 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% similarity. By "similarity" includes "identity".

In a related embodiment, enabled herein is an isolated antibody which binds to A β ₁₋₄₀ or C-terminally elongated forms thereof or covalently linked multimers thereof, the antibody comprising a modified form of Fab 2286 wherein a mature heavy chain variable region comprises a modified amino acid sequence set forth in SEQ ID NO:1 with the proviso that the amino acid residue at position 50 is not glutamic acid (E), wherein the numbering of the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) represents amino acid residues 47 to 50 or comprising one or more amino acid substitutions, additions and/or deletions to the amino acid sequence of SEQ ID NO: 1.

In an embodiment, the amino acid residue at position 50 is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the amino acid residue at position 50 is a basic amino acid such as arginine, lysine or histidine. In an embodiment, the amino acid residue at position 50 is alanine. In an embodiment, the amino acid residue at position 50 is arginine.

The present disclosure is instructional on a Fab 2286-like antibody comprising a mature heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO:2 wherein the Fab 2286-like antibody binds to A β ₁₋₄₀ and to C-terminally elongated forms thereof.

Other examples of the heavy chain variable regions are found in SEQ ID NOs:9 and 11.

Reference to "C-terminally elongated forms" of $A\beta_{1-40}$ includes $A\beta_{1-42}$, $A\beta_{1-43}$, $A\beta_{3-42}$, $A\beta_{4-42}$, $A\beta_{pyroGlu3-42}$ and $A\beta_{pyroGlu11-42}$.

Enabled herein is an isolated polynucleotide encoding an amino acid sequence of a mature heavy chain variable region of an antibody which binds to $A\beta_{1-40}$ and C-terminally elongated forms thereof or covalently linked multimers thereof, the antibody comprising a modified form of Fab 2286 wherein a mature heavy chain variable region comprises the amino acid sequence set forth in SEQ ID NO: 1 wherein the amino acid at position 50 is not glutamic acid or comprising one or more other amino acid substitutions, additions and/or deletions to the amino acid sequence of SEQ ID NO: 1. The numbering of the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) represents amino acid residues 47 to 50. In an embodiment, the amino acid at position 50 is selected from the list constituting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the amino acid at position 50 is a basic amino acid such as arginine, lysine or histidine. In an embodiment, the amino acid at position 50 is alanine. In an embodiment, the amino acid residue at position 50 is arginine. Hence, in an embodiment, the polynucleotide encodes SEQ ID NO:2. Cells and vectors comprising the polynucleotide are also enabled herein. Polynucleotide encoding SEQ ID NOs:9 and 11 are also contemplated herein as well as polynucleotide sequence which have at least 90% identity to the nucleotide sequence encoding each of SEQ ID NO:2, 9 or 11 or a nucleic acid capable of hybridizing under medium or high stringency conditions to the nucleotide sequence encoding SEQ ID NO:2, 9 or 11 provided the nucleotide sequence encodes Glu50Arg substitution. In an embodiment, the nucleotide sequence is SEQ ID NO:18 or a nucleotide having at least 90% identity to SEQ ID NO:18 or a nucleotide sequence which hybridizes to the complement of SEQ ID NO: 18 under medium or high stringency conditions. In relation to a nucleotide sequence, "at least 90%" means 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100%.

The term "similarity" as used herein includes exact identity between compared sequences at the nucleotide or amino acid level. Where there is non-identity at the nucleotide level, "similarity" includes differences between sequences which result in different amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. Where there is non-identity at the amino acid level, "similarity" includes amino acids that are nevertheless related to each other at the structural, functional, biochemical and/or conformational levels. In an embodiment, nucleotide and sequence comparisons are made at the level of identity rather than similarity.

Terms used to describe sequence relationships between two or more polynucleotides or polypeptides include "reference sequence", "comparison window", "sequence similarity", "sequence identity", "percentage of sequence similarity", "percentage of sequence identity", "substantially similar" and "substantial identity". A "reference sequence" is at least 12 but frequently 15 to 18 and often at least 25 or above, such as 30 monomer units, inclusive of nucleotides and amino acid residues, in length. Because two polynucleotides may each comprise (1) a sequence (i.e. only a portion of the complete polynucleotide sequence) that is similar

between the two polynucleotides, and (2) a sequence that is divergent between the two polynucleotides, sequence comparisons between two (or more) polynucleotides are typically performed by comparing sequences of the two polynucleotides over a "comparison window" to identify and compare local regions of sequence similarity. A "comparison window" refers to a conceptual segment of typically 12 contiguous residues that is compared to a reference sequence. The comparison window may comprise additions or deletions (i.e. gaps) of about 20% or less as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Optimal alignment of sequences for aligning a comparison window may be conducted by computerized implementations of algorithms (e.g. GA β , BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package Release 7.0, Genetics Computer Group, 575 Science Drive Madison, Wis., USA) or by inspection and the best alignment (i.e. resulting in the highest percentage homology over the comparison window) generated by any of the various methods selected. Reference also may be made to the BLAST family of programs as for example disclosed by Altschul et al. (1997) *Nucl. Acids. Res.* 25:3389. A detailed discussion of sequence analysis can be found in Unit 19.3 of Ausubel et al. (*In: Current Protocols in Molecular Biology*, John Wiley & Sons Inc. 1994-1998).

The terms "sequence similarity" and "sequence identity" as used herein refers to the extent that sequences are identical or functionally or structurally similar on a nucleotide-by-nucleotide basis or an amino acid-by-amino acid basis over a window of comparison. Thus, a "percentage of sequence identity", for example, is calculated by comparing two optimally aligned sequences over the window of comparison, determining the number of positions at which the identical nucleic acid base (e.g. A, T, C, G, I) or the identical amino acid residue (e.g. Ala, Pro, Ser, Thr, Gly, Val, Leu, Ile, Phe, Tyr, Trp, Lys, Arg, His, Asp, Glu, Asn, Gln, Cys and Met) occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison (i.e., the window size), and multiplying the result by 100 to yield the percentage of sequence identity. For the purposes of the present invention, "sequence identity" will be understood to mean the "match percentage" calculated by the DNASIS computer program (Version 2.5 for windows; available from Hitachi Software engineering Co., Ltd., South San Francisco, Calif., USA) using standard defaults as used in the reference manual accompanying the software. Similar comments apply in relation to sequence similarity.

Reference to medium stringency includes and encompasses from at least about 16% v/v to at least about 30% v/v formamide and from at least about 0.5 M to at least about 0.9 M salt for hybridization, and at least about 0.5 M to at least about 0.9 M salt for washing condition. Reference to high stringency includes and encompasses from at least about 31% v/v to at least about 50% v/v formamide and from at least about 0.01 M to at least about 0.15 M salt for hybridization, and at least about 0.01 M to at least about 0.15 M salt for washing conditions. In general, washing is carried out $T_m = 69.3 + 0.41 (G+C) \%$ (Marmur and Doty (1962) *J. Mol. Biol.* 5:109). However, the T_m of a duplex DNA decreases by 1° C. with every increase of 1% in the number of mismatch base pairs (Bonner and Laskey (1974) *Eur. J. Biochem.* 46:83). Formamide is optional in these hybridization conditions. Accordingly, particularly preferred levels of stringency are defined as follows: low stringency is 6xSSC

buffer, 0.1% w/v SDS at 25°-42° C.; a moderate stringency is 2×SSC buffer, 0.1% w/v SDS at a temperature in the range 20° C. to 65° C.; high stringency is 0.1×SSC buffer, 0.1% w/v SDS at a temperature of at least 65° C.

The Fab 2286-like antibody disclosed herein is useful in the treatment of a subject with a disease or condition associated with or characterized by the presence of pathogenic or toxic forms of A β species. The C-terminally elongated A β species may also be in the form of cerebral deposits. Such diseases or conditions include Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]). Generally, the subject is a human although for testing purposes, non-human mammalian subjects may be tested. In an example, a humanized antibody is used in humans and a murinized form is used for mice or rats. In an embodiment, the subject is a companion animal such as a dog or cat (using caninized and felinized antibodies, respectively). See, for example, SEQ ID NOs:9 and 11.

Hence, the instant disclosure teaches a method for the treatment or prophylaxis of a condition associated with or characterized by the presence of a pathogenic form of A β comprising a C-terminally elongated species of A β , the method comprising administering to a subject in need of treatment an effective amount of an antibody comprising a mature heavy chain having a modified amino acid sequence set forth in SEQ ID NO: 1 with the proviso that the amino acid residue at position 50 is not glutamic acid, using a numbering system where the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) is at positions 47 to 50 or an amino acid sequence with one or more other amino acid substitutions, additions and/or deletions thereto. In an embodiment, the amino acid at position 50 is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the amino acid at position 50 is a basic amino acid such as arginine, lysine or histidine. In an embodiment, the amino acid at position 50 is alanine. In an embodiment, the amino acid at position 50 is arginine.

In an embodiment, the instant disclosure teaches a method for the treatment or prophylaxis of a condition associated with or characterized by the presence of a pathogenic form of A β comprising a C-terminally elongated species of A β , the method comprising administering to a subject in need of treatment an effective amount of an antibody comprising a mature heavy chain having the amino acid sequence set forth in SEQ ID NO:2 or an amino acid sequence with one or more amino acid substitutions, additions and/or deletions thereto with the proviso that the amino acid residue at position 50 is R, using a numbering system where the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) is at positions 47 to 50.

The effective amount of antibody includes an amount sufficient for microglia to be stimulated to facilitate removal of pathogenic forms of A β to which the antibody binds. Generally, the antibody does not substantially provoke an inflammatory response, the Fab 2286-like antibody promotes clearance of pathogenic A β species. This can be via microglial removal of A β in the brain or via a shift in the equilibrium of A β from the brain to the circulatory blood system ("peripheral sink").

Enabled herein is a method of treating or ameliorating symptoms of a neurological condition selected from Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma, stroke and HCHWA-D or other adverse event involving amyloidosis in a subject, the method comprising administering to the subject, an effective amount of a Fab 2286-like antibody comprising a mature heavy chain variable region having a modified amino acid sequence set forth in SEQ ID NO: 1 with the proviso that the amino acid residue at position 50 is not glutamic acid (E) using a numbering system where the amino acid sequence WIGE of Fab 2286 (SEQ ID NO: 1) is at positions 47 to 50 or having one or more other amino acid substitutions, additions and/or deletions thereto. In an embodiment, the amino acid substitution may also be referred to as Glu50Xaa in the heavy chain variable region of Fab 2286. In an embodiment, Xaa is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, Xaa is a basic amino acid residue such as arginine, lysine or histidine. In an embodiment, Xaa is alanine. In a particular embodiment, Xaa is arginine (i.e. the substitution is Glu50Arg).

The term "Fab 2286-like antibody" means that the Fab 2286 antibody has undergone a modification to the mature heavy chain variable region to substitute the E in the amino acid sequence WIGE at positions 47 to 50 to an R (i.e. WIGR) [Glu50Arg] or another amino acid [Glu50Xaa], wherein Xaa is not glutamic acid. One or more other amino acid substitutions, additions and/or deletions may also be made to the mature heavy chain or a light chain. The Fab 2286-like antibody, however, binds to A β_{1-40} and C-terminally elongated forms thereof such as A β_{1-42} and A β_{1-43} . In addition, the Fab 2286-like antibody may be conjugated to any light and heavy chain constant region scaffold. The original 2286 antibody (V_L+C_L; V_H+C_H) is referred to as Mab 2286. In an embodiment, the Fab 2286-like antibody is conjugated to heavy and light chain constant regions of any antibody.

The antibody may also bind to covalently bound multimers of these A β species.

In an embodiment, the present disclosure teaches a method for the treatment or prophylaxis of Alzheimer's disease in a subject, the method comprising administering to the subject an effective amount of an Fab 2286-like antibody having a modification to its mature heavy chain to substitute an E in the amino acid sequence WIGE to X (WIGX), wherein X is any amino acid except glutamic acid. In an embodiment, X is R (WIGR).

One or more other amino acid substitutions, additions and/or deletions may also be made to the heavy or light chain. In one embodiment, the V_L+C_L comprises an amino acid sequence as set forth in SEQ ID NO:9.

The inventors analyzed the molecular basis of antibody engagement of A β epitopes. The 3D Fab structure (Fab 2286) was determined to near atomic resolution of the variable domain of Mab 2286. Fab 2286 is a chimera comprising the mouse variable region of Mab 2286 transplanted onto a human scaffold. Fab 2286 is V_H and V_L from Mab 2286 and C_H and C_L from a human template. The analysis revealed a hydrophobic cavity in the Fab 2286 binding site in which the C-terminal region of A β_{1-40} was modeled. Data further revealed a buried terminal carboxyl location that excluded cross reactivity with C-terminally

elongated A β species. Modification of the glutamic acid (E) at residue 50 by the substitution to another amino acid residue in the mature heavy chain variable region enables binding to the C-terminally elongated A β species.

Reference to an "antibody" includes an immunoglobulin molecule which binds to a specific target. In the case of the Fab 2286-like antibody of the instant disclosure, the specific target is the C-terminal end of A β_{1-40} or C-terminally elongated A β species such as A β_{1-42} and A β_{1-43} as well as A β_{3-42} , A β_{4-42} , A $\beta_{\text{pyroGlu3-42}}$ and A $\beta_{\text{pyroGlu11-42}}$. The immunoglobulin binds to the C-terminal end of the elongated pathogenic forms of A β . Further covered herein are fragments of antibodies such as Fab, Fab', F(ab')₂ and Fv fragments, single chain (ScFv) forms, mutants, fusion proteins comprising a portion of the Fab 2286-like antibody and synthetically modified derivatives. In an embodiment, the Fab 2286-like antibody is conjugated to h κ 1 light chain and hG1 heavy chain constant regions.

As used herein, an "effective dosage" or "effective amount" of the Fab 2286-like antibody or a pharmaceutical composition comprising same is an amount sufficient to effect beneficial or desired results. For prophylactic use, beneficial or desired results include results such as eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. Such a disease is a disease or condition associated with pathogenic forms of A β . Examples include Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]). For therapeutic use, beneficial or desired results include clinical results such as inhibiting, suppressing or reducing the formation of amyloid plaques, reducing, removing, clearing amyloid plaques, improving cognition, reversing or slowing cognitive decline, sequestering or increasing soluble A β peptide circulating in biological fluids, decreasing one or more symptoms resulting from the disease (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes presenting during development of the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, enhancing effect of another medication, delaying the progression of the disease, and/or prolonging survival of patients. An effective dosage can be administered in one or more administrations. For purposes of the present disclosure, an effective dosage of antibody, or pharmaceutical composition is an amount sufficient to accomplish prophylactic or therapeutic treatment either directly or indirectly. As is understood in the clinical context, an effective dosage of the Fab 2286-like antibody or pharmaceutical composition comprising same may or may not be achieved in conjunction with another drug, compound, or pharmaceutical composition. Thus, an "effective dosage" may be considered in the context of administering one or more therapeutic agents, and a single agent may be considered to be given in an effective amount if, in conjunction with one or more other agents, a desirable result may be or is achieved.

As used herein, "treatment" or "treating" is an approach for obtaining beneficial or desired results including clinical results. For purposes of this disclosure, beneficial or desired

clinical results include, but are not limited to, one or more of the following: inhibiting, suppressing or reducing the formation of amyloid plaques, reducing, removing, or clearing amyloid plaques, improving cognition, reversing or slowing cognitive decline, sequestering soluble A β peptide circulating in biological fluids, reducing A β peptide (including soluble, oligomeric and deposited) in a tissue (such as brain), inhibiting, slowing and/or reducing accumulation of A β peptide in the brain, inhibiting, slowing and/or reducing toxic effects of A β peptide in a tissue (such as brain), decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, delaying the progression of the disease, and/or prolonging survival of patients. Reference to disease includes Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]).

As used herein, "delaying" development of Alzheimer's disease means to defer, hinder, slow, retard, stabilize, and/or postpone development of the disease or its symptoms. This delay can be of varying lengths of time, depending on the history of the disease and/or individual being treated. As is evident to one skilled in the art, a sufficient or significant delay can, in effect, encompass prevention, in that the individual does not develop the disease. A method that "delays" development of Alzheimer's disease is a method that reduces probability of disease development in a given time frame and/or reduces extent of the disease in a given time frame, when compared to not using the method. Such comparisons are typically based on clinical studies, using a statistically significant number of subjects.

"Development" of Alzheimer's disease means the onset and/or progression of Alzheimer's disease within an individual. Alzheimer's disease development can be detectable using standard clinical techniques. However, development also refers to disease progression that may be initially undetectable. For purposes of this disclosure, progression refers to the biological course of the disease state, in this case, as determined by a standard neurological examination, patient interview, or may be determined by more specialized testing such as the detection or monitoring of biological markers of the disease. A variety of these diagnostic tests include, but not limited to, neuroimaging, detecting alterations of levels of specific proteins in the serum or cerebrospinal fluid (e.g. amyloid peptides and Tau), computerized tomography (CT), and magnetic resonance imaging (MRI). "Development" includes occurrence, recurrence, and onset. As used herein "onset" or "occurrence" of Alzheimer's disease includes initial onset and and/or recurrence.

As used herein, administration "in conjunction" includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions. As used herein, administration in conjunction is meant to encompass any circumstance wherein an anti-A β antibody and another agent are administered to an individual, which can occur simultaneously and/or separately. As further enabled herein, it is understood that an anti-A β Fab 2286-like antibody and optionally the other agent can be administered at different dosing frequencies or intervals. For example, an anti-A β antibody can be administered weekly, while the other agent can be administered less frequently. It is understood that the anti-A β

antibody and the other agent can be administered using the same route of administration or different routes of administration.

An "individual" (alternatively referred to as a "subject") is a mammal, including a human. Other mammals include, but are not limited to, farm animals (such as cows), sport animals, companion animals (such as cats, dogs, horses), primates, mice and rats.

The Fab 2286-like antibody may be formulated in a pharmaceutical composition with a pharmaceutically acceptable carrier, "pharmaceutically acceptable carrier" includes any material which, when combined with an active ingredient, allows the ingredient to retain biological activity and is non-reactive with the subject's immune system. Examples include, but are not limited to, any of the standard pharmaceutical carriers such as a phosphate buffered saline solution, water, emulsions such as oil/water emulsion, and various types of wetting agents. Diluents for aerosol or parenteral administration include phosphate buffered saline or normal (0.9%/w/v) saline. Compositions comprising such carriers are formulated by well known conventional methods (see, for example, *Remington's Pharmaceutical Sciences*, 18th edition, A. Gennaro, ed., Mack Publishing Co., Easton, Pa., 1990; and *Remington, The Science and Practice of Pharmacy* 20th Ed. Mack Publishing, 2000).

The Fab 2286-like antibody may include additional modifications which include functionally equivalent antibodies which do not significantly affect their properties and variants which have enhanced or decreased activity and/or affinity. For example, the amino acid sequence of the heavy or light variable region of the Fab 2286-like antibody may be mutated to obtain an antibody with a desired binding affinity to A β ₁₋₄₂ of A β ₁₋₄₃ peptide. Modification of polypeptides is routine practice in the art and need not be described in detail herein. Examples of modified polypeptides include polypeptides with conservative substitutions of amino acid residues, one or more deletions or additions of amino acids which do not significantly deleteriously change the functional activity, or use of chemical analogs.

Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to an epitope tag. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody of an enzyme or a polypeptide which increases the serum half-life of the antibody or which provides a particular functionality.

Substitution variants have at least one amino acid residue in the antibody molecule removed and a different residue inserted in its place. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but Fc alterations are also contemplated. Conservative substitutions are shown in Table 3 under the heading of "conservative substitutions".

TABLE 3

Amino Acid substitutions		
Original Residue	Conservative Substitutions	Exemplary Substitutions
Ala (A)	Val	Val; Leu; Ile
Arg (R)	Lys	Lys; Gln; Asn

TABLE 3-continued

Amino Acid substitutions		
Original Residue	Conservative Substitutions	Exemplary Substitutions
Asn (N)	Gln	Gln; His; Asp; Lys; Arg
Asp (D)	Glu	Glu; Asn
Cys (C)	Ser	Ser; Ala
Gln (Q)	Asn	Asn; Glu
Glu (E)	Asp	Asp; Gln
Gly (G)	Ala	Ala
His (H)	Arg	Asn; Gln; Lys; Arg
Ile (I)	Leu	Leu; Val; Met; Ala; Phe; Norleucine
Leu (L)	Ile	Norleucine; Ile; Val; Met; Ala; Phe
Lys (K)	Arg	Arg; Gln; Asn
Met (M)	Leu	Leu; Phe; Ile
Phe (F)	Tyr	Leu; Val; Ile; Ala; Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	Tyr	Tyr; Phe
Tyr (Y)	Phe	Trp; Phe; Thr; Ser
Val (V)	Leu	Ile; Leu; Met; Phe; Ala; Norleucine

Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining: (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation; (b) the charge or hydrophobicity of the molecule at the target site; or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties: (1) Non-polar: Norleucine, Met, Ala, Val, Leu, Ile; (2) Polar without charge: Cys, Ser, Thr, Asn, Gln; (3) Acidic (negatively charged): Asp, Glu; (4) Basic (positively charged): Lys, Arg; (5) Residues that influence chain orientation: Gly, Pro; and (6) Aromatic: Trp, Tyr, Phe, His. As taught herein, the Fab 2286-like antibody comprises a substitution of E to an R in the sequence WIGE in the heavy chain variable region of Fab 2286 (wild type).

Non-conservative substitutions are made by exchanging a member of one of these classes for another class.

Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant cross-linking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability, particularly where the antibody is an antibody fragment such as an Fv fragment.

Amino acid modifications can range from changing or modifying one or more amino acids to complete redesign of a region, such as the variable region. Changes in the variable region can alter binding affinity and/or specificity.

Modifications also include glycosylated and nonglycosylated polypeptides, as well as polypeptides with other post-translational modifications, such as, for example, glycosylation with different sugars, acetylation, and phosphorylation. Antibodies are glycosylated at conserved positions in their constant regions (Jefferis and Lund (1997) *Chem. Immunol.* 65:111-128; Wright and Morrison (1997) *TibTECH* 15:26-32). The oligosaccharide side chains of the immunoglobulins affect the protein's function (Boyd et al. (1996) *Mol. Immunol.* 32:1311-1318; Wittwe and Howard (1990) *Biochem.* 29:4175-4180) and the intramolecular interaction between portions of the glycoprotein, which can affect the conformation and presented three-dimensional surface of the glycoprotein (Jefferis and Lund (1997) supra; Wyss and Wagner (1996) *Current Opin. Biotech.* 7:409-416). Oligosaccharides may also serve to target a given

glycoprotein to certain molecules based upon specific recognition structures. Glycosylation of antibodies has also been reported to affect antibody-dependent cellular cytotoxicity (ADCC).

Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine, asparagine-X-threonine, and asparagine-X-cysteine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

The glycosylation pattern of antibodies may also be altered without altering the underlying nucleotide sequence. Glycosylation largely depends on the host cell used to express the antibody. Since the cell type used for expression of recombinant glycoproteins, e.g. antibodies, as potential therapeutics is rarely the native cell, variations in the glycosylation pattern of the antibodies can be expected (see, e.g. Hse et al. (1997) *J. Biol. Chem.* 272:9062-9070).

The methods taught herein use antibodies (including pharmaceutical compositions comprising the antibodies) that specifically bind to an A β peptide at the C-terminal end. The antibodies are further characterized by any (one or more) of the following characteristics: (a) suppresses formation of amyloid plaques in a subject; (b) reduces amyloid plaques in a subject; (c) treats, prevents, ameliorates one or more symptoms of Alzheimer's disease; (d) improves cognitive function. The antibodies described herein may exhibit a desirable safety profile, for example, the compositions of Fab 2286-like antibody do not cause significant or unacceptable levels or have a reduced level of any one or more of: bleeding in the brain vasculature (cerebral hemorrhage); meningoencephalitis (including changing magnetic resonance scan); elevated white blood count in cerebral spinal fluid; central nervous system inflammation.

The Fab 2286-like antibodies, polynucleotides encoding amino acid chains of the Fab 2286-like antibody, and pharmaceutical compositions described herein can be used in methods for treating, preventing and inhibiting the development of a disease characterized by aberrant deposition of A β peptide in the brain of a subject. The methods comprise administering to the subject an effective amount of the Fab 2286-like antibody that specifically binds to the A β peptide or the A β peptide deposit or a polynucleotide encoding a chain of the antibody.

Without limiting the present disclosure to any one hypothesis or mode of action, the Fab 2286-like antibody can act directly in the brain to induce microglial-mediated removal of A β or via the "peripheral sink" route. According to the latter mode of action, the Fab 2286-like antibody does not need to cross the blood-brain barrier to act, but rather, binds A β in the blood and shift the equilibrium of A β from the

CNS to the plasma, where A β can be degraded (DeMattos et al. (2001) *Proc Natl Acad Sci USA* 98:8850-8855).

The Fab 2286-like antibodies, polynucleotides encoding an amino acid chain in the antibody, and pharmaceutical compositions described herein can be used in methods for treating, preventing and inhibiting the development of Alzheimer's disease and other diseases associated with altered A β or APP expression, or accumulation or deposit of A β peptide (collectively termed "A β -associated diseases"), such as Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, glaucoma, vascular disorder caused by deposit of A β peptide in blood vessels (such as stroke and HCHWA-D). Such methods comprise administering the Fab 2286-like antibodies or a pharmaceutical composition comprising same to the subject. In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, Alzheimer's disease (or other A β -associated disease) in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay the outset of the disease, including biochemical, histological and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In therapeutic applications, compositions or medicaments are administered; to a patient suspected of, or already suffering from such a disease in amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histological and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease.

The present disclosure teaches a method of delaying development of a symptom associated with Alzheimer's disease (or other A β -associated disease) in a subject comprising administering an effective dosage of a pharmaceutical composition comprising a Fab 2286-like antibody described herein to the subject. Symptoms associated with Alzheimer disease includes, but not limited to, abnormalities of memory, problem solving, language, calculation, visuospatial perception, judgment, and behavior.

This disclosure enables a method of inhibiting or suppressing the formation of amyloid plaques and/or A β accumulation in a subject comprising administering an effective dose of a pharmaceutical composition comprising a Fab 2286-like antibody to the subject. In an embodiment, the amyloid plaques are in the brain of the subject. In an embodiment, the amyloid plaques are in the cerebral vasculature of the subject. In an embodiment, the A β accumulation is in the circulatory system of the subject.

The instant disclosure teaches a method of reducing amyloid plaques and/or reducing or slowing A β accumulation in a subject comprising administering an effective dose of a pharmaceutical composition comprising an Fab 2286-like antibody to the subject an Fab 2286-like antibody. In an embodiment, the amyloid plaques are in the brain of the subject. In an embodiment, the amyloid plaques are in the cerebral vasculature of the subject. In an embodiment, the A β accumulation is in the circulatory system of the subject.

Further taught herein is a method of removing or clearing amyloid plaques and/or A β accumulation in a subject comprising administering an effective dose of a pharmaceutical composition comprising an Fab 2286-like antibody to the subject an Fab 2286-like antibody. In an embodiment, the amyloid plaques are in the brain of the subject. In some embodiments, the amyloid plaques are in the cerebral vasculature of the subject. In an embodiment, the A β accumulation is in the circulatory system of the subject.

The present disclosure is instructional for a method of reducing A β peptide in a tissue (such as brain), inhibiting and/or reducing accumulation of A β peptide in a tissue (such as brain), and inhibiting and/or reducing toxic effects of A β peptide in a tissue (such as brain) in a subject comprising administering an effective dose of a pharmaceutical composition comprising an Fab 2286-like antibody to the subject an Fab 2286-like antibody. A β polypeptide may be in soluble, oligomeric, or deposited form. An oligomeric form of A β may be composed of 2 or more (e.g. 2 to 50) A β polypeptides, which can be a mixture of inter alia A β ₁₋₄₀, A β ₁₋₄₂ and A β ₁₋₄₃ peptides as well as A β ₃₋₄₂, A β ₄₋₄₂, A β _{pyroGlu3-42} and A β _{pyroGlu11-42} and the like.

The present disclosure teaches a method of improving cognition or reversing cognitive decline associated with diseases associated with amyloid deposit of A β in a subject, such as Alzheimer's disease, comprising administering an effective dosage of a pharmaceutical composition comprising an Fab 2286-like antibody to the subject an Fab 2286-like antibody.

Effective dosages and schedules for administering the Fab 2286-like antibody may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage of antibody that must be administered will vary depending on, for example, the mammal that will receive the antibody, the route of administration, the particular type of antibody used and other drugs being administered to the mammal. Guidance in selecting appropriate doses for antibody is found in the literature on therapeutic uses of antibodies, e.g., Handbook of Monoclonal Antibodies, Ferrone et al. eds. Noyes Publications, Park Ridge, N.J. (1985) ch. 22:303-357; Smith et al. (1977) *Antibodies in Human Diagnosis and Therapy*, Haber et al. eds., Raven Press, New York:365-389. A typical daily dosage of the antibody used alone might range from about 1 μ g/kg to up to 100 mg/kg of body weight or more per day, depending on the factors mentioned above. Generally, any of the following doses may be used: a dose of at least about 50 mg/kg body weight; at least about 10 mg/kg body weight; at least about 3 mg/kg body weight; at least about 1 mg/kg body weight; at least about 750 μ g/kg body weight; at least about 500 μ g/kg body weight; at least about 250 μ g/kg body weight; at least about 100 μ g/kg body weight; at least about 50 μ g/kg body weight; at least about 10 μ g/kg body weight; at least about 1 μ g/kg body weight, or more, is administered. Antibodies may be administered at lower doses or less frequent at the beginning of the treatment to avoid potential side effect. Administration may be by any route including intracerebral and intravenous. The latter is useful in promoting a "peripheral sink" effect.

In an embodiment, more than one antibody is present. Such compositions may contain at least one, at least two, at least three, at least four, at least five different antibodies which bind to different epitopes on A β or which bind to the same epitope but with different binding avidities.

The Fab 2286-like antibody may also be administered to a mammalian subject in combination with effective amounts of one or more other therapeutic agents. The antibody may be administered sequentially or concurrently with the one or more other therapeutic agents. The amounts of antibody and therapeutic agent depend, for example, on what type of drugs are used, the pathological condition being treated, and the scheduling and routes of administration but would generally be less than if each were used individually.

Following administration of antibody to the mammal, the mammal's physiological condition can be monitored in various ways well known to the skilled practitioner. In an

embodiment, the mammal is a human, companion animal, simian animal or laboratory test animal such as a mouse or rat.

The instant disclosure further teaches articles of manufacture and kits containing materials useful for treating pathological conditions described herein, such as Alzheimer's disease or other A β -associated diseases (such as Down's syndrome, Parkinson's disease, multi-infarct dementia, mild cognitive impairment, cerebral amyloid angiopathy, glaucoma, vascular disorder caused by deposit of A β peptide in blood vessels [such as stroke and HCHWA-D]), or detecting or purifying A β or APP. The article of manufacture comprises a container with a label. Suitable containers include, for example, bottles, vials, and test tubes. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition having an active agent which is effective for treating pathological conditions or for detecting or purifying A β or APP. The active agent in the composition is an Fab 2286-like antibody and may further include an anti-Fab 2286-like antibody labeled with a reporter molecule or enzyme. The label on the container indicates that the composition is used for treating pathological conditions such as Alzheimer's disease or detecting or purifying A β or APP, and may also indicate directions for either in vivo or in vitro use.

The present disclosure also provides kits comprising the Fab 2286-like antibody and/or polynucleotides encoding amino acid chains of Fab 2286-like antibody. In an embodiment, the kit disclosed herein comprises the container described above. In an embodiment, the kit comprises the container described above and a second container comprising a buffer. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, syringes, and package inserts with instructions for performing any methods described herein (such as methods for treating Alzheimer's disease, and methods for inhibiting or reducing accumulation of A β peptide in the brain). In kits to be used for detecting or purifying A β or APP, the antibody is typically labeled with a detectable marker, such as, for example, a radioisotope, fluorescent compound, bioluminescent compound, a chemiluminescent compound, metal chelator or enzyme.

In an embodiment, described herein is a composition for use in any of the methods described herein, whether in the context of use as a medicament and/or use for manufacture of a medicament. Hence, taught herein is a use of an antibody which binds to A β ₁₋₄₀ or C-terminally elongated forms thereof or covalently linked multimers thereof, the antibody comprising a modified Fab 2286 wherein a mature heavy chain comprises a modified amino acid sequence set forth in SEQ ID NO: 1 with the proviso that the amino acid residue at position 50 is not glutamic acid, wherein the numbering of the amino acid sequence WIGE in Fab 2286 (SEQ ID NO:1) represents amino acid residues 47 to 50 or comprising one or other more amino acid substitutions, additions and/or deletions to the amino acid sequence of SEQ ID NO:2 in the manufacture of a medicament for the treatment of pathogenic A β disease. In an embodiment, the amino acid is selected from the list consisting of alanine, arginine, asparagine, aspartic acid, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine, pyrrolysine and selenocysteine. In an embodiment, the amino acid is a basic amino acid residue such as arginine, lysine or histidine. In an embodiment, the amino acid is alanine. In a particular embodiment, the amino acid is

arginine. In an embodiment, the A β disease is selected from the list consisting of Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]).

Another aspect enabled herein is a method of detecting a toxic form of A β in a sample from a subject, said method comprising identifying binding between the A β form and Fab 2286-like antibody.

Diagnostic applications include the detection of Alzheimer's disease, Down's syndrome, cognitive impairment or memory loss, Parkinson's disease, multi-infarct dementia, cerebral amyloid angiopathy, glaucoma and a vascular disorder caused by pathogenic A β peptide in blood vessels (e.g. stroke and hereditary cerebral hemorrhage with amyloidosis-Dutch type [HCHWA-D]) as well as pre-eclampsia.

In an embodiment, the Fab 2286-like antibody is used to detect Alzheimer's disease, HCHWA-D or pre-eclampsia.

Any immunoassay may be used to capture and/or directly identify a toxic form of A β . Immunoassays are binding assays. Certain immunoassays contemplated herein are the various types of enzyme linked immunosorbent assays (ELISAs) and radioimmunoassays (RIA) known in the art. However, it will be readily appreciated that detection is not limited to such techniques, and Western blotting, dot blotting, FACS analyses, and the like may also be used.

In an embodiment, the assay is capable of generating quantitative results.

The Fab 2286-like antibody is either labeled with a reporter molecule or the Fab 2286-like antibody is used to bind to or capture the A β and a second antibody (directed to Fab 2286-like antibody or a portion of A β) labeled with a reporter molecule is used to detect an Fab 2286-like antibody-A β complex.

The Fab 2286-like antibody can be used to screen for or capture toxic forms of A β . Techniques for the assays contemplated herein are known in the art and include, for example, sandwich assays and ELISA.

It is within the scope of this invention to include any second antibodies (monoclonal, polyclonal or fragments of antibodies or synthetic antibodies) directed to Fab 2286-like antibody. Both types of antibodies may be used in detection assays or the Fab 2286-like antibody may be used with a commercially available anti-immunoglobulin antibody.

Both polyclonal and monoclonal antibodies specific for Fab 2286-like antibody are obtainable by immunization with Fab 2286-like antibodies or antigenic fragments thereof and either type is utilizable for immunoassays. The methods of obtaining both types of sera are well known in the art. Polyclonal sera are less preferred but are relatively easily prepared by injection of a suitable laboratory animal with an effective amount of the Fab 2286-like antibody, or antigenic parts thereof, collecting serum from the animal and isolating specific sera by any of the known immunoabsorbent techniques. Although antibodies produced by this method are utilizable in virtually any type of immunoassay, they are generally less favoured because of the potential heterogeneity of the product.

The use of monoclonal antibodies in an immunoassay is particularly preferred because of the ability to produce them in large quantities and the homogeneity of the product. The preparation of hybridoma cell lines for monoclonal antibody production derived by fusing an immortal cell line and lymphocytes sensitized against the immunogenic prepara-

tion can be done by techniques which are well known to those who are skilled in the art.

Another aspect of the present invention contemplates, therefore, a method for detecting a toxic form of A β in a biological sample from a subject. The method comprising contacting the biological sample with Fab 2286-like antibody for a time and under conditions sufficient for an antibody-polypeptide complex to form, and then detecting the complex. The Fab 2286-like antibody may be labeled with a reporter molecule and this is detected or a labeled anti-Fab 2286-like antibody or generic labeled anti-immunoglobulin antibody is employed to detect the Fab 2286-like antibody-A β complex.

A biological sample includes a blood or cerebral spinal fluid sample. For the detection of pre-eclampsia, the sample is urine.

A "labeled" antibody means an antibody labeled with a reporter molecule. By "reporter molecule" as used in the present specification, is meant a molecule which, by its chemical nature, provides an analytically identifiable signal which allows the detection of antigen-bound antibody. Detection may be either qualitative or quantitative. The most commonly used reporter molecules in this type of assay are either enzymes, fluorophores or radionuclide containing molecules (i.e. radioisotopes) and chemiluminescent molecules. In the case of an enzyme immunoassay, an enzyme is conjugated to a second antibody, generally by means of glutaraldehyde or periodate. As will be readily recognized, however, a wide variety of different conjugation techniques exist, which are readily available to the skilled artisan.

Alternately, fluorescent compounds, such as fluorescein and rhodamine, may be chemically coupled to antibodies without altering their binding capacity. When activated by illumination with light of a particular wavelength, the fluorochrome-labeled antibody adsorbs the light energy, inducing a state to excitability in the molecule, followed by emission of the light at a characteristic colour visually detectable with a light microscope. The fluorescent labeled antibody is allowed to bind to a Fab 2286-like antibody-A β complex. After washing off the unbound reagent, the remaining tertiary complex is then exposed to the light of the appropriate wavelength the fluorescence observed indicates the presence of the hapten of interest. Immunofluorescence and EIA techniques are both very well established in the art. However, other reporter molecules, such as radioisotope, chemiluminescent or bioluminescent molecules, may also be employed. In an embodiment, taught herein is a dipstick comprising Fab 2286-like antibody immobilized thereon for use in detecting toxic forms of A β in a biological sample. In an embodiment, the sample is urine and the condition diagnosed is pre-eclampsia.

EXAMPLES

Aspects of the present disclosure are now further described by the following non-limiting Examples.

Materials and Methods

Antibody Cloning and Expression and Purification

A chimeric antibody was produced incorporating light and heavy chain variable domains of the anti-A β murine monoclonal antibody, Mab 2286, described by Rosenthal et al. (US Patent Application Nos. 2004/046512 and 2007/0160616) conjugated, respectively, to human kappa 1 immunoglobulin (hKappa) light chain and gamma-1 immunoglobulin (hG1) heavy chain constant regions. Amino acid

sequences for the Fab region of the light (V_L+C_L) [SEQ ID NO:3] and heavy (V_H+C_H) [SEQ ID NO: 1] chains of Fab 2286 are shown in Table 4.

Heavy and light chain constructs were each cloned into the pcDNA 3.1 vector (Invitrogen). Plasmids were transformed into *E. coli* (DH5-alpha, Invitrogen) for amplification under ampicillin selection and purified with a PureLink (Trade Mark) HiPure Plasmid Megaprep Kit (Invitrogen) according to the manufacturer's instructions. Recombinant expression plasmids were then transfected into FreeStyle (Trade Mark) 293-F cells to allow expression of the recombinant chimeric antibody.

FreeStyle (Trade Mark) 293-F cells were cultured in FreeStyle expression medium (Invitrogen), and maintained at 37° C. with an atmosphere of 8% v/v CO₂. Transient transfections were performed using 293Fectin transfection reagent (Invitrogen) according to the manufacturer's instructions. Cultures of 1000 mL were shaken in a Cellbag (Trade Mark) 2 L (flow rate: 0.1-0.15 Lpm) on a bioreactor system. Cultures were supplemented with 0.1% Pluronic F68 (Invitrogen) and 0.5% Lucratone Lupin (Millipore) 4 and 24 hours post transfection, respectively, and the rocking angle adjusted to 9° after 24 hours. Six days post-transfection the cell culture supernatants were harvested by centrifugation at 1000×g.

Harvested conditioned media was filtered through a 0.22 µm filter and applied to a MabSelect SuRe HiTrap Protein A HP Column (5 ml, Hitrap, GE Life Sciences, Sweden) previously equilibrated with PBS. Antibody was eluted with 0.1 M sodium citrate pH 3.5 and collected into 10% v/v final volume of 3.0 M Tris pH 8.0. Antibody was buffer exchanged into PBS using a HiLoad desalting column 26/10 (GE Life Sciences, Sweden) and concentrated to 1 mg/mL with a centrifugal concentrator (Amicon ultra, 50 kDa MWCO) for storage at -80° C.

For expression and purification of Fab2286-like antibody fragment, synthetic DNA cloned into pcDNA3.1 expression vectors were obtained from Genscript for expression of the heavy and light chains. The N-terminal signal peptides were incorporated for the heavy chain (MGWSWIFLFL VSGTG-GVLSE) and light chain (MESQTQVLMS LLFWVS-GTCG). The heavy chain was expressed as a Hexa-histidine tagged construct, with the tag at the C-terminus of the chain. DNA constructs were transformed into DH5α *E. coli* for amplification under ampicillin selection and purified with a PureLink (Trade Mark) HiPure Plasmid Megaprep Kit (Invitrogen) according to the manufacturer's instructions. Recombinant expression plasmids were then co-transfected into FreeStyle (Trade Mark) 293-F cells (Invitrogen) to allow expression of the recombinant antibody fragment.

293-F cells were cultured in FreeStyle expression medium (Invitrogen), and maintained at 310 K with an atmosphere of 8% v/v CO₂. The expression was performed in 4 L batches in a Certomat Ct plus incubator (Sartorius) by doing co-transfection of 1×10⁶ cells/mL with both DNA and 293Fectin transfection reagent (Invitrogen) according to the manufacturer's instructions. Cultures were supplemented with 5 mL/L of 10% v/v Pluronic F68 (Invitrogen), 5 mg/L Lucratone Lupin (Millipore) 4 hours post transfection, and 5 mg/L of glucose 2 days post-transfection. The cell culture supernatants were harvested by centrifugation at 500×g, and the media collected for purification.

4 L of harvested media was concentrated to 200 mL by tangential flow filtration system (Millipore Proflux M12). The concentrated media was centrifuged at 20000×g for 30 minutes before being purified by immobilised-metal affinity chromatography. The supernatant containing Fab was incu-

bated for 1 hour with equilibrated Ni-NTA affinity resin (Qiagen). The mixture was washed 4 times with 20 mM Tris pH 8.0, 150 mM NaCl and 20 mM imidazole. The protein of interest was eluted with 20 mM Tris pH 8.0, 150 mM NaCl and 500 mM imidazole. Eluted sample was further purified by size exclusion chromatography with a HiLoad Superdex 200 20/60 run in PBS on an AKTApurifier (GE Healthcare). Fractions were concentrated to 2 mg/mL with a centrifugal concentrator (Amicon ultra, 10 kDa MWCO).

Purification of Fab

Fab fragments were prepared using the Immunopure Fab Preparation kit (Pierce Biotechnology). IgG (10 mg) was digested with 0.5 mL of the 50% w/v immobilized papain slurry overnight at room temperature. The immobilized papain was removed by centrifugation (3000×g for 3 minutes) and the supernatant containing the digested sample was loaded onto a 1 mL HiTrap Protein A column (GE Life Sciences, Sweden). Fractions containing the clean Fab fragments were pooled and dialyzed over night in 20 mM HEPES pH 7.0. The dialyzed sample was concentrated with a Centriprep-10 centrifugal concentrator to 4 mg/mL (10 kDa MWCO, Millipore). The digestion and purification steps were monitored by SDS-PAGE.

Amyloid-β Peptides

Peptides corresponding to residues 1-28 (Aβ28), 1-40 (Aβ40) and 1-42 (Aβ42) of the amyloid-β sequence (1-DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA-42) [SEQ ID NO:6] were obtained from commercial sources: Aβ28 (AnaSpec, San Jose, Calif., USA); Aβ40 and Aβ42 (GenicBio BioTech Co., Shanghai, China). Each of the Aβ peptides was resuspended in 100% (v/v) 2,2,2-trifluoroethanol (TFE) and aliquoted to give 100 µg per Eppendorf tube. All aliquots were freeze-dried for 4 hours and stored at -80° C. until required.

SDS-PAGE and Western Blot

Aβ peptides (100 µg) were dissolved in 2 µL of 10 µM NaOH, then made up to a volume of 100 µL with PBS. Samples of Aβ40 and Aβ42 were prepared in advance and aged at room temperature for either 24 hours or 21 days. A 1:1 molar ratio of Aβ40 and Aβ42 was also prepared and allowed to age overnight before running on SDS-PAGE. Aβ28 was used as a negative control and prepared on the day along with fresh samples of Aβ40 and Aβ42.

An aliquot of 0.5 µg of all aged and freshly prepared Aβ peptides in non-reducing Lamelli loading dye was run on 12% w/v SDS polyacrilamide gel (NuPage; Invitrogen). Gels were run in triplicate, one gel was Coomassie stained with InstantBlue (Expedon) and the other two were transferred for 1 hour at 4° C. to nitrocellulose membrane (Millipore).

Following Western transfer, the nitrocellulose membrane was blocked with 3% w/v BSA in PBS with 0.1% v/v Tween-20 (PBS-T) overnight at 4° C. The membrane was incubated with either Mab 2286 (1:5000) or Mab WO2 (1:2000) in 3% v/v BSA, PBS-T for 1 hour at room temperature. It was then washed four times in PBS-T for 15 minutes each. The blot was then incubated with 1:5000 Protein A-HRP (Millipore) in PBS-T for 1 hour at room temperature. It was washed a further four times in PBS-T for 15 minutes. The membrane was incubated with SuperSignal West Pico chemiluminescent (Pierce) for 5 minutes, exposed for 1 minute to X-ray film (Super RX; Fuji) and developed. Crystallization

Initial crystallization screening resulted in small needles of crystals that were subsequently optimized by a combination of the Additive Screen (Hampton Research) and grid screening varying pH and PEG concentration. The best

crystals were grown in 100 mM citric acid pH 5.5, 20% v/v 2-propanol and 20% w/v PEG 4000. All crystals were grown using by vapor diffusion using 2 μ L hanging drops at 22° C. Crystals were soaked in a cryoprotectant (mother liquor plus 15% v/v glycerol) for several minutes prior to mounting in a N₂ stream at 100 K.

Structure Determination

Diffraction data from a single crystal of Fab 2286 were collected on the MXI beamline of the Australian Synchrotron (Melbourne, Australia) with an ADSC Quantum 210r detector. Data collection were controlled using Blue-Ice software (McPhillips et al. (2002) *J. Synchrotron Radiat* 9:401-406). All diffraction data were acquired from one crystal frozen at 100 K. Data collection statistics are summarized in Table 5.

Accession Numbers

The coordinates for the Fab 2286 structure have been deposited in the Protein Data Bank under the accession number 3U0W.

TABLE 4

Amino acid sequences of the chimeric Fab 2286 light and heavy chains

Light Chain (hKppa)

DIQMTQTTSSLSASLGDRTISCSASQGISNYLWFFQKPDGTVKLLIY
YTSLSLHSGVPSRFRSGSGTDYSLTISNLEPEDIAITYYCQYRKLPTF
GGGKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCCLNFFPREAKVQ
WKVDNALQSGNSQESVTEQDSKSTYLSLSLTLSKADYEKHKVYACEV
THQGLSSPVTKSFNRGEC (SEQ ID NO: 3)

Heavy Chain (hG1)

EVKLLSEGGGLVQPGGSLKISCAASGFDFSRVWMMNWRQAPGKGLEWIG
EINPDSSTINYPPLKDKFIIIRDNAKNTLYLQMSKVRSEDTAIYYCAR
QMGYWGQTTTLTVSSASTKGPSVPLAPSSKSTSGGTAALGCLVKDYFP
EPVTVSWNSGALTSVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYIC
NVNHKPSNTKVKDKVEPKSC (SEQ ID NO: 10)

Example 1

Western Blot Analysis

Antibody recognition of A β peptides is confounded by not only the range of different length peptides produced, but by their propensity to form a range of higher order complexes. In order to explore the specificity of Mab 2286, a number of different A β peptide preparations were examined. These included denatured and freshly dissolved A β 28, A β 40 and A β 42, which would be mostly monomeric in solution. The peptides were allowed to oligomerize/aggregate both in isolation and as a mixture of the two most common peptides, A β 40 and A β 42, for 24 hours to 21 days. These were then analyzed by SDS-PAGE and detected by Coomassie stain (FIG. 1A), Western Blot with chimeric anti-A β Mab 2286 as the primary antibody (FIG. 1B) and Western Blot with murine anti-A β Mab WO2 as the primary antibody (FIG. 1C).

Mab WO2 was used as the reference antibody as the 3-D A β recognition epitope was previously determined, which involves the free side chains of A β ₂₋₇ (Miles et al. (2008) *J Mol Biol* 377:181-192). Consistent with the epitope recognition of Mab WO2, specific bands corresponding to A β 28, A β 40 and A β 42 were detected as well as a range of higher molecular weight A β species observed for all of the aged peptides (FIG. 1C), indicating that the aging protocol used was sufficient to generate a number of oligomeric forms.

By contrast, quite strikingly, Mab 2286 was highly selective and sensitive in detecting monomeric A β 40, with no

cross-reactivity with any of the other aggregated forms apart from a minor A β 40 dimeric species (FIG. 1B, Lane 2). There was no cross-reactivity with either the A β 28 or A β 42 sample (FIG. 1B, Lane 6 and 4 respectively). Interestingly, no monomeric A β 40 was detected in the sample that had been left to age for 21 days (FIG. 1B Lane 1), suggesting that all monomeric A β 40 had reacted to form oligomers/aggregates. The preparation comprised a mixture of A β 40 and A β 42 and was allowed to age overnight. Under these conditions the level of monomeric A β 40 detected by Mab 2286 was significantly reduced (FIG. 1B Lane 5) relative to freshly prepared A β 40 alone (the starting concentration of monomeric A β 40 is the same as the sample in Lane 2) indicating that it has formed higher molecular weight oligomers in the presence of A β 42.

Rosenthal and co-workers showed that Mab 2286 is specific for A β terminating at residue 40. They also showed that single point alanine mutations across the terminal residues (35-MVGGVV-40 [SEQ ID NO:8]) modulated binding affinity (Rosenthal et al. US Patent Application No. 2007/0160616). Unlike the N-terminal region of A β , the C-terminal region is hydrophobic. When combined, these data indicate that Mab 2286 recognises the hydrophobic side chains of A β 40 at the C-terminus that are predominantly available in the monomeric state or available for recruitment from perhaps dimers or higher order complexes.

Example 2

Structure of Fab 2286

High resolution (2 Å) structure of a chimeric form of an antigen binding fragment of Mab 2286 [Fab 2286], was determined (Table 5). The structure of Fab 2286 reveals that it is a typical immunoglobulin Fab heavy/light chain heterodimer with an elbow angle of 185.2°. The putative A β binding groove in the Fab2286 crystal structure was calculated to be approximately 1900 Å³ using Fred Receptor (v2.2.5, OpenEye Scientific Software, Inc., <http://www.eyesopen.com>).

TABLE 5

Data collection and refinement statistics	
Crystal	Fab 2286
Data collection	
Space group	P2 ₁ 2 ₁
Unit-cell parameters	
a (Å)	64.0
b (Å)	81.3
c (Å)	108.5
Maximum resolution (Å)	2.0
Total observations	437605
Unique reflections used	39062
Redundancy	11.7 (5.0) ^[a]
Completeness (%)	95.7 (71.7)
I/ σ _I	27.9 (2.3)
R _{SYM} (%) ^[b]	9.7 (83.1)
Final refinement statistics	
Resolution (Å)	2.0
Total no. of atoms	3511
No. of waters	304
R-factor (%) ^[c]	18.9
R-free (%) ^[d]	23.5
r.m.s.d. bond lengths (Å)	0.021
r.m.s.d. bond angles (°)	2.0

TABLE 5-continued

Data collection and refinement statistics	
Mean B-factor	
Protein m.c. (s.c.)	28.4 (33.1)
Solvent	41.2
Ramachandran regions (%)	
Favored	90.0
Allowed	9.7
Disallowed	0.3

^(a)The values in parentheses are for the highest resolution bin.

^(b) $R_{SYM} = \sum_{hkl} \sum_i |I_i - \langle I \rangle| / \langle I \rangle$, where I_i is the intensity for the i th measurement of an equivalent reflection with indices h, k, l .

^(c)R-factor = $\sum ||F_{obs}| - |F_{calc}|| / \sum |F_{obs}|$, where F_{obs} and F_{calc} are the observed and calculated structure factor amplitudes respectively.

^(d)R-free was calculated with 5% of the diffraction data that were selected randomly and not used throughout refinement.

Example 3

Generation of Fab 2286-Like Antibody

Mab 2286 exhibits specificity for the carboxyl terminus of $A\beta_{40}$ and shows no significant cross reactivity with C-terminally extended peptides such as $A\beta_{42}$ and $A\beta_{43}$. Antibody binding to $A\beta_{40}$ is not inhibited by $A\beta_{38}$ in competitive binding assays, and binding is similarly modulated by conservative alanine single point mutations across the mapped residues (35-MVGGVV-40 [SEQ ID NO:8]).

The structure of chimeric Fab 2286 determined herein reveals a mechanism explaining specificity for the $A\beta_{40}$ C-terminus and involves extensive contacts with the $A\beta_{40}$ Val39 side chain buried in a hydrophobic pocket at the antibody interface. With the side chain of $A\beta_{40}$ Val39 anchored in this way, the side chain of Val40 makes further hydrophobic contacts such that the free carboxyl moiety can hydrogen bond with Asn35(H). C-terminally extended $A\beta$ peptides would lose this potential hydrogen bond and the additional residues would force the ligand backbone to adopt a conformation that is incompatible with the $A\beta$ binding site.

The Western Blot analysis shown in FIG. 1 indicates that, unlike the N-terminus, this hydrophobic C-terminal epitope is buried in oligomeric/aggregated synthetic $A\beta_{40}$, as only bands correlating with monomer and dimer molecular weights are detected by the binding fragment of Mab 2286, i.e. Fab 2286. It is concluded that the epitope of $A\beta$ recognized by Fab 2286 is buried in higher order oligomeric assemblies is consistent with the crystal structures of the peptide reported by Colletier et al. (2011) *Proc Natl Acad Sci USA* 108(41):16938-43. In that work, fibril-like structures of the peptide were shown to exhibit parallel and anti-parallel β -sheet stacked forms. The two steric zippers reported identify "knobs-in-holes" type packing, with Val39 (and Ile41) constituting the buried 'knobs' accommodated by the "holes" enabled by the presence of the glycine residues.

It is known that amyloid plaques in the Alzheimer's diseased brain are mainly composed of C-terminally extended species such as $A\beta_{42}$ and $A\beta_{43}$, and these represent significant pathogens in Alzheimer's disease. According to the "peripheral sink" hypothesis (DeMattos et al. (2001) supra), anti- $A\beta$ antibodies need not cross the blood brain barrier to act, but rather, bind $A\beta$ in the blood and shift the equilibrium of $A\beta$ from the CNS to the plasma, where $A\beta$ can be degraded.

A His-tagged fusion of Fab 2286 chains was generated using human kappa-1 immunoglobulin (hkappa-1) light chain and gamma-1 immunoglobulin (hG1) heavy chain

constant regions. In the Fab 2286, Glu50 is mutated to Arg (Glu50Arg) on the heavy chain. The Fab 2286-like antibody produced recognized the hydrophobic C-terminal epitope available in monomeric forms of $A\beta$, but one which can also accommodate C-terminally extended $A\beta$ peptides which have a greater propensity to aggregate or nucleate/accelerate oligomerization.

Synthetic DNA constructs corresponding to heavy and light chains of Fab 2286 were ordered from Genscript and subcloned into the pcDNA 3.1 vector (Invitrogen) for expression in mammalian cells. A single point mutation was introduced into the heavy chain protein sequence based on a computational analysis of Fab 2286, an antibody fragment that has only low micromolar affinity for amyloid beta peptide terminating at residue 40. The mutant Fab was designed to recognize $A\beta_{1-40}$. It also was able to bind toxic C-terminally extended peptides including $A\beta_{1-42}$ and $A\beta_{1-43}$. The resultant mutant Fab not only bound the longer length peptides but also did so with low nanomolar affinity. FIGS. 2 and 3 compare the $A\beta$ binding profiles between Fab 2286 and the Fab 2286-like antibody Glu50Arg Fab mutant against synthetic $A\beta$ species and against $A\beta$ species in brain tissue affected by familial and sporadic Alzheimer's disease respectively.

Example 4

Generation of Fab 2286-Like Antibody on a Murine Scaffold

A murinized form of the Fab 2286-like antibody was generated. To murinized the antibody, the following sequences were employed:

Monoclonal antibody 2286 (Mab 2286) nucleic acid sequence: heavy chain [variable domain and constant domain 1 (CH1)]; [SEQ ID NO: 12]:
gagggtgaagcttctcgagctcggaggtggcctggcgagcctggaggatc
cctgaaactctcctgtgcagcctcaggattcgatttagtagatactgga
tgaattgggtccggcaggctccagsgaaaggctagaatggattggagaa
atataatccagatagcagtagcagataaaactatacgcctctcctaaggataa
attcatcatctccagagacaacgcaaaaatacagctgtacctgcaaatga
gcaaagtgagatctgaggacacagcccttattactgtgcaagacaatg
ggctactggggccaaggcaccactctcacagctcctcagccaaaacgac
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acagtgacctggaactctggatcctgtccagcggtgtgcacacctccc
agctgtcctgcagctctgacctctacactctgagcagctcagtgactgtcc
cctccagcactggcccagcgagaccatcacctgcaacgttggccaccgg
gccagcagcaccagggtggacaagaaaattgtgccagggtattgt
and the light chain: (SEQ ID NO: 14):
gatatacagatgacacagactacatcctcctctgtctgctctctgggaga
cagagtcaccatcagttgcagtgcaagtcagggtcattagcaattatttaa
actggtttcagcagaaaccagatggaactgnaaactcctgatctattaca
catcaagtttacactcaggagtcaccatcaagggtcagtgccagtggtct

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gggacagattattctctcaccatcagcaacctggaacctgaagatattgc
 cacttactattgtcagcagtataggaagcttccgtacacgttcggaggg
 gggaccaagctggaataaaaacgggctgatgctgcaccaactgtatccat
 cttcccaccatccagtgagcagttaacatctggagggtgcctcagtcgtgt
 gcttcttgaacaacttctaccccaagacatcaatgtcaagtggaagatt
 gatggcagtgaaacgacaaaatggcgctcctgaacagttggactgatcagga
 cagcaaaagacagcacctacagcatgagcagcaccctcacgttgaccaagg
 acgagatgaacgacataaacagctatacctgtgagggcactcacaagaca
 tcaacttcaccattgtcaagagcttcaacaggaatgagtgt

The Mab 2286 amino acid sequence: heavy chain
 [variable domain and constant domain 1 (CH)];
 [SEQ ID NO: 13]:
 EVKLLLESGGGLVQPGGSLKLSAASGFDFSRYSRYWNNVVRQAPGKLEWIGE
 INPDSSITYNTPSLKDKFIIISRDNAKNTLYLQMSKVRSEDTALYYCARQM
 GYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPEPV
 TVTWNSSGSLSSGVHTFPFVAVLQSDLYTLSSSVTVPSSTWPSSETVTCNVAHP
 ASSTKVDKKI VPRDC

and the light chain; (SEQ ID NO: 15):
 DIQMTQTSSLSASLGRVITISCSASQGISNYLNWFQKPDGTVKLLIYY
 TSSLHSGVPSRFSGSGSDYSLTISNLEPEDIATYYCQYRKLPTFGG
 GTKLEIKRADAAPTVISIFPPSSEQLTSGGASVVCFLNMFYDPKINVKWKI
 DGSERQNGVLNWDQSDKSTYSMSSTLTLTKDEYERHNSYTCETHKT
 STSPIVKSFNRNEC

The Fc portion from a murine gamma heavy chain (Gen-
 Bank: AAA75163.1) was added to the heavy chain Fab
 portion sequence. The synthetic DNA construct was:

Gene name: MurineHeavyA_OPT, Length: 1391 bp,
 Sequence (SEQ ID NO: 16):
 GAGGTCAAACCTGCTGGAGAGTGGAGGGGACTGGTGCAGCCAGCGGGT
 CACTGAAGCTGAGCTGCGCCGCTTCCGGCTTCGACTTTTCCCGGACTG
 GATGAATTGGGTGAGACAGGCTCCCGAAAAGGCCTGGAGTGGATCGGG
 GAAATTAATCCTGATAGCTCCACCATCAACTACACACCAAGTCTGAAGG
 ACAAATTCATCATTTACCGGATAACGCAAAGAATACTCTGTATCTGCA
 GATGTCTAAAGTGCAGAGTGGAGACACCGCACTGTACTATTGTGCAAGA
 CAGATGGGATACTGGGGACAGGGAACCACTGACCGTGTCTAGTGCTA
 AGACTACCCCTCCACAGTGTATCCTCTGGCACCTGGCTCCGCAGCACAG
 ACCAATTCTATGGTGACACTGGGCTGTCTGGTCAAGGGTACTTCCCTG
 AGCCAGTCACAGTACTTGAACAGCGGCAGCCTGTCAAGCGGCGTGCA
 CACCTTCTCTGCGCTCTGCAGAGGATCTGTATACACTGTCTCTAGT
 GTCACCTGTGCCCTCAAGCACTGGCCTTCCGAGACCGTGACATGCAACG
 TCGCCCATCTGCTTCTCTACAAAGGTGGACAAGAAAATCGTCCACG
 AGATTGCGCTGTAAACCATGCATTTGTACTGTCCCGAAGTGAAGTTCAG
 TCTTCATCTTTCCACCAAGCCAAAAGACGTGTGACTATTACCCTGAC

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ACCCAAGGTCACATGCGTGGTCTGGATATCAGCAAAGACGATCCCGAG
 GTGCAGTTCTCCTGGTTTGTTCGACGATGTGGAAGTGCACACAGCCAGA
 5 CTCAGCCAGGGAGGAAACAGTTCAATTCTACCTTTTCGCTCTGTGAGTGA
 GCTGCCTATTATGCATCAGGACTGGCTGAATGGAAGGAATTCAAATGC
 AGAGTGAACCTCTGCTGCATTTCCCGCTCCTATCGAGAAGACTATTAGCA
 10 AGACCAAAGGCAGGCTTAAAGCCCCACAGGTGTACACAAATCCCTCCACC
 CAAGGAACAGATGGCTAAGGATAAAGTGAGCCTGACATGTATGATCACT
 GACTTCTTTCCCGAGGATATTACCGTGAATGGCAGTGGAACGGGCGAGC
 15 CCGCAGAGAACTATAAGAATACACAGCCTATTATGGACACTGATGGATC
 ATACTTCGTGTATAGCAAGCTGAACGTCACAGAAATCTAATTGGGAAGCC
 GGCAACACTTTTAQCCTGTAGTGTGCTGCAATGAAGGACTGCATAACCAT
 20 CATACTGAAAAGTCACTGTCTCATTCCACAGGCAAA

and the amino acid sequence (SEQ ID NO: 17):
 EVKLLLESGGGLVQPGGSLKLSAASGFDFSRYSRYWNNVVRQAPGKLEWIG
 EINPDSSITYNTPSLKDKFIIISRDNAKNTLYLQMSKVRSEDTALYYCAR
 25 QMGYWGQGTTLTVSSAKTTPPSVYPLAPGSAAQTNSMVTLGCLVKGYFPE
 PVTVTWNSSGSLSSGVHTFPFVAVLQSDLYTLSSSVTVPSSTWPSSETVTCN
 V AHPASSTKVDKKI VPRDCGCKPCICTVPEVSSVFI PPPKPKDVLITLTL
 30 PKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTQPREEQFNSTFRSVSE
 LPIMHQDWLNGKEPKCRVNSAAFPAIEKTSKTKGRPKAPQVYTI PPP
 KEQMAKDKVSLTCMIDTFPEPDI TVWQWNGQPAENYKNTQPI MDTDGSY
 35 FVYSKLVQKSNWEAGNTFTCSVLHEGLHNHHTKSLSHSPGK

The mutant Fab 2286-like antibody (with the Glu to Arg
 substitution) was derived from mutagenesis of that sequence
 yielding:

Variant name, Murine Heavy B, Variant sequence
 (SEQ ID NO: 18):
 GAGGTCAAACCTGCTGGAGAGTGGAGGGGACTGGTGCAGCCAGCGGGT
 45 ACTGAAGCTGAGCTGCGCCGCTTCCGGCTTCGACTTTTCCCGGATACTGG
 ATGAATTGGGTGAGACAGGCTCCCGAAAAGGCCTGGAGTGGATCGGGCG
 TATTAATCCTGATAGCTCCACCATCAACTACACACCAAGTCTGAAGGACA
 50 AATTCATCATTTACCGGATAACGCAAAGAATACTCTGTGATCTGCAGAT
 GTCTAAAGTGCAGAGTGGAGACACCGCACTGTACTATTGTGCAAGACAGA
 TGGGATACTGGGGACAGGGAACCACTGACCGTGTCTAGTGCTAAGACT
 55 ACCCTCCAGCGTGTATCCTCTGGCACCTGGCTCCGCAGCACAGACCAA
 TTCTATGGTGACACTGGGCTGTCTGGTCAAGGGGTACTTCCCTGAGCCAG
 TCACAGTACTTGAACAGCGGCAGCCTGTCAAGCGGCGTGACACCTTT
 60 CCTGCGCTCTGCAGAGCGATCTGTATACACTGTCTCTAGTGTCACTGT
 GHCCCTCAAGCACCTGGCCTTCCGAGACCGTGACATGCAACGTCGCCCCAT
 CCTGCTTCTCTACAAAGGTGGACAAGAAAATCGTCCACAGGATTGCGG
 CTGTAACCATGCATTTGTACTGTCCCGAAGTGAAGTTCAGTCTTCATCT
 65 TTCCACCAAGCCAAAAGACGTGTGACTATTACCCTGACACCAAGGTC

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ACATGCGTGGTCTGGATATCAGCAAAGACGATCCCGAGGTGCAGTTCTC
 CTGGTTTGTTCGACGATGTCGAAGTGACACAGCCCAGACTCAGCCCAGGG
 AGGAACAGTTCAATTCTACCTTTTCGCTCGTGAAGTGCAGTGCCTATTATGC
 ATCAGGACTGGCTGAATGGAAGGAATTCAAATGCAGAGTGAACCTGCT
 GCATTTCCCGCTCCTATCGAGAAGACTATTAGCAAGACCAAAGGCAGGCC
 TAAAGCCCCACAGGTGTACACAATCCCTCCACCCAAAGGAACAGATGGCTA
 AGGATAAAGTGAGCCTGACATGTATGATCACTGACTTCTTTCCCGAGGAT
 ATTACCGTGAATGGCAGTGGAAACGGGCAGCCCGCAGAGAAGTATAAGAA
 TACACAGCCTATATTGGACACTGATGGATCATACTTCGCTGATAGCAAGC
 TGAACGTCAGAAATCTAATTGGGAAGCCGGCAACACTTTTACCTGTAGT
 GTGCTGCATGAAGACTGCATAACCATCATACTGAAAAGTCACTGTCTCA
 TTCACCAGGCCAAA

and the amino acid sequence (SEQ ID NO: 19)
 EVKLLLESGGGLVQPPGSLKLSAASGFDFSRYSRYWNNVVRQAPGKLEWIG
 EINPDSSTINITYPSLKDKFII SRDNAKNTLYLQMSKVRSEDTALYYCAR
 QMGYWGQTTTLTVSSAKTTPPSVYPLAPGSAAQNSMVTLGCLVKGYFPE
 PVTVTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVPSSTWPSSETVTCNV
 AHPASSTKVDKIVPRDCGCKPCICTVPEVSSVFI FPPKPKDVLITITLT
 PKVTCVVVDISKDDPEVQFSWFVDDVEVHTAQTPREEQFNSTFRSVSE
 LPIMHQDLNGLKEFKRNVNSAAPPAPIEKTISKTKGRPKAPQVYTI PPP
 KEQMAKDKVSLTLCMTDFPEDI TVWQWNGQPAENYKNTQPIMDTGSY
 FVYSKLVNQSNEAGNTFTCSVLHEGLHNNHTEKLSLHSPGK

The synthetic DNA light chain construct is:

Murine Light OPT, Length: 725 bp, Sequence
 (SEQ ID NO: 20):
 GATATTCAGATGACTCAGACTACTTCTTCCCTGTCTGCAAGTCTGGGGGA
 CCGAGTGACAATCTCATGCAGCGCCTCCAGGGAATTTCCAACCTACCTGA
 ATTGGTTCCAGCAGAAGCCTGATGGCACAGTGAAACTGCTGATCTACTAT
 ACTAGTCCCTGCACAGTGGGGTCCCATCAAGATTTCTGGAAAGTGGCTC
 AGGGACCGACTATAGCCTGACAAATCTCCAACCTGGAGCCAGAAGATATTG
 CCACTTACTATTGCCAGCAGTACCGAAGCTGCCCTATACTTTCCGCGGG
 GGAACCAAGCTGGAGATCAAAGAGCTGACGCGCTCCCAACCGTGAGCAT
 TTTTCCCTCTTAGTGAACAGCTGACCTCTGGCGGGCAAGTGTGGTCT
 GTTTCCTGAACAACTTCTACCCATAAGACATCAACGTGAAGTGGAAAATT
 GATGGAAGCGAGAGGCAGAACGGCGTCTGAAATTCCTGGACCGACCAGGA
 TAGCAAGGACTCCACATATCTATGTCAAGCACCCCTGACACTGACTAAAG
 ATGAGTACGAACGCCATAATAGCTATACATGTGAAGCTACTCATAAGACC
 TCTACCTCTCTATTGTGAAATCTTTTAACCGAAATGAATGT

and the amino acid sequence (SEQ ID NO: 21):
 DIQMTQTSSLSASLGRVTISCSASQGISNYLNWFQQKPDGTVKLLIYY
 TSSLHSGVPSRFSGSGSDYSLTISNLEPEDIATYYCQQYRKLPTYPFG

-continued

GTKLEIKRADAAPT VSI FPPSSEQLTSGGASVVCFLNMFYPKD INVKWKI
 DGSERQNGVLNSWTDQDSKDYSTYSMSSTLTLTKDEYERHNSYTCEATHKT
 STSPIVKSFNFNEC

The murinized Fab 2286-like antibody is used in animal model studies.

Example 5

Animal Model

15 Mouse model protocols for the evaluation of Fab 2286-
 like antibody are established. Efficacy of the antibody is
 tested in Tg2576 mice which over express A β ₄₀ or other
 mice which over express elongated forms of A β . To test Fab
 2286-like antibody or a derivative thereof, APP/PS1 mice are
 used. An anti-A β drug efficacy protocol such as by Kenche
 et al. (2013) *Angewandte Chemie International Edition*
 52:3374-3378 is a reliable template to formulate a study
 design. These mice generate significant quantities of both
 A β ₄₀ and A β ₄₂ in the brain and are amyloid positive at
 around 7-8 months, cognitively impaired at 9-10 months.
 Mice are purchased at ~3 months of age from The Jackson
 Laboratory, Bar Harbor, Me., USA, and are allowed 2
 months for acclimatization at a housing facility. APP/PS1
 mice are randomly assigned to different treatment groups,
 (e.g. 10 mg/kg Fab 2286-like antibody, 10 mg/kg vehicle
 control). Behavioral assessment and tissue analysis follow
 protocols established by Kenche et al. (2013) supra. Treat-
 ment begins at 5 months before amyloid deposition has
 begun in three arms of 15 animals each (based on a Power
 statistical analysis for a significant outcome). Behavioral
 assessments begin at 7 months of age by Y-maze and water
 platform techniques as described by Kenche et al. (2013)
 supra. Weekly dosing is administered by oral gavage until 10
 months of age when tissue is collected and subject to
 immunohistochemical analysis and examined for differential
 A β ₄₀/A β ₄₂ profiles by SELDI-TOF MS and other IP/MS
 methods.

Example 6

Detection of Pre-Eclampsia

45 Urine samples are obtained from healthy women, healthy
 pregnant women and pregnant women diagnosed with pre-
 eclampsia. The levels and species of A β are determined in
 each group. In an alternative, cerebral spinal fluid is tested.
 However, a urine sample is far less invasive. Fab 2286-like
 antibody is used to either capture the A β species which is
 then detected using a labeled anti-immunoglobulin antibody
 or the Fab 2286-like antibody is labeled itself. It is expected
 that pregnant women with pre-eclampsia will exhibit
 elevated levels of brain-derived toxic A β species comprising
 C-terminally elongated forms.

The Fab 2286-like antibody may also be used in the
 development of a dipstick or other similar approach to detect
 toxic A β in urine of pregnant women.

Example 7

Generation of Modified Fab 2286 Heavy Chains

65 Using the techniques disclosed herein or known to the
 skilled artisan, a range of substitutions is made at amino acid
 residue 50 in the heavy chain variable region of Fab 2286.

The amino acid sequence set forth in SEQ ID NO:2 comprises an arginine in place of a glutamic acid at residue 50. This creates the amino acid sequence comprising WIGR.

Other substitutions are selected from WIGA, WIGR, WIGN, WIGD, WIGC, WIGQ, WJGG, WIGH, WIGI, WIGL, WIGK, WIGM, WIGF, WIGP, WIGS, WIGT, WIGW, WIGY, WIGV, WIGO and WIGU.

An antibody comprising any of these substitutions from glutamic acid are readily tested as disclosed herein for the ability to bind to A β ₁₋₄₀ or C-terminally elongated forms.

Those skilled in the art will appreciate that the disclosure described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the disclosure contemplates all such variations and modifications. The disclosure also enables all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of the steps or features or compositions or compounds.

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SEQUENCE LISTING

<160> NUMBER OF SEQ ID NOS: 21

<210> SEQ ID NO 1

<211> LENGTH: 215

<212> TYPE: PRT

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Amino acid sequence of heavy chain of Fab 2286 antibody

<400> SEQUENCE: 1

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60

Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110

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Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 115 120 125

Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
 130 135 140

Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
 145 150 155 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
 165 170 175

Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
 180 185 190

Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
 195 200 205

Lys Ile Val Pro Arg Asp Cys
 210 215

<210> SEQ ID NO 2
 <211> LENGTH: 215
 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of heavy chain of Fab
 2286-like antibody

<400> SEQUENCE: 2

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60

Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110

Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 115 120 125

Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
 130 135 140

Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
 145 150 155 160

Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
 165 170 175

Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
 180 185 190

Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
 195 200 205

Lys Ile Val Pro Arg Asp Cys
 210 215

<210> SEQ ID NO 3
 <211> LENGTH: 214
 <212> TYPE: PRT

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<213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of light chain (hkappa)

<400> SEQUENCE: 3

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
 1 5 10 15
 Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile Ser Asn Tyr
 20 25 30
 Leu Asn Trp Phe Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
 35 40 45
 Tyr Tyr Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
 50 55 60
 Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro
 65 70 75 80
 Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Arg Lys Leu Pro Tyr
 85 90 95
 Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala
 100 105 110
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly
 115 120 125
 Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala
 130 135 140
 Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
 145 150 155 160
 Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser
 165 170 175
 Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr
 180 185 190
 Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser
 195 200 205
 Phe Asn Arg Gly Glu Cys
 210

<210> SEQ ID NO 4
 <211> LENGTH: 28
 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of Abeta1-28

<400> SEQUENCE: 4

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
 1 5 10 15
 Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys
 20 25

<210> SEQ ID NO 5
 <211> LENGTH: 40
 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of Abeta1-40

<400> SEQUENCE: 5

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
 1 5 10 15
 Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
 20 25 30

-continued

Gly Leu Met Val Gly Gly Val Val
 35 40

<210> SEQ ID NO 6
 <211> LENGTH: 42
 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of Abeta1-42

<400> SEQUENCE: 6

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 1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
 20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala
 35 40

<210> SEQ ID NO 7
 <211> LENGTH: 43
 <212> TYPE: PRT
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<400> SEQUENCE: 7

Asp Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys
 1 5 10 15

Leu Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile
 20 25 30

Gly Leu Met Val Gly Gly Val Val Ile Ala Thr
 35 40

<210> SEQ ID NO 8
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 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of Abeta35-40

<400> SEQUENCE: 8

Met Val Gly Gly Val Val
 1 5

<210> SEQ ID NO 9
 <211> LENGTH: 437
 <212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Amino acid sequence of murinized Fab 2286-like
 antibody heavy chain conjugated to human gamma-1 immunoglobulin
 (hG1) heavy constant region with Glu50Arg substitution

<400> SEQUENCE: 9

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30

Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

Gly Arg Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60

-continued

Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95
 Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110
 Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 115 120 125
 Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
 130 135 140
 Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
 145 150 155 160
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
 165 170 175
 Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
 180 185 190
 Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
 195 200 205
 Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val
 210 215 220
 Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val
 225 230 235 240
 Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile
 245 250 255
 Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val
 260 265 270
 Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser
 275 280 285
 Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu
 290 295 300
 Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala
 305 310 315 320
 Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro
 325 330 335
 Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys
 340 345 350
 Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr
 355 360 365
 Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr
 370 375 380
 Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu
 385 390 395 400
 Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser
 405 410 415
 Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser
 420 425 430
 His Ser Pro Gly Lys
 435

<210> SEQ ID NO 10

<211> LENGTH: 216

<212> TYPE: PRT

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Amino acid sequence of chimeric Fab2286

-continued

antibody heavy chain variable region

<400> SEQUENCE: 10

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Ile Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60
 Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110
 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser
 115 120 125
 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp
 130 135 140
 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr
 145 150 155 160
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr
 165 170 175
 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln
 180 185 190
 Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp
 195 200 205
 Lys Lys Val Glu Pro Lys Ser Cys
 210 215

<210> SEQ ID NO 11

<211> LENGTH: 222

<212> TYPE: PRT

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Amino acid sequence of chimeric Fab 2286-like antibody heavy chain variable region

<400> SEQUENCE: 11

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15
 Ser Leu Lys Ile Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30
 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45
 Gly Arg Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60
 Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80
 Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Ile Tyr Tyr Cys
 85 90 95
 Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110
 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser

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tataagaata cacagcctat tatggacact gatggatcat acttctgtgta tagcaagctg 1200
aacgtccaga aatctaattg ggaagccggc aacactttta cctgtagtgt gctgcatgaa 1260
ggactgcata accatcatac tgaaaagtca ctgtctcatt caccaggcaa a 1311

<210> SEQ ID NO 17
<211> LENGTH: 437
<212> TYPE: PRT
<213> ORGANISM: artificial
<220> FEATURE:
<223> OTHER INFORMATION: Murine heavy A_Opt amino acid sequence

<400> SEQUENCE: 17

Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
1 5 10 15
Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
20 25 30
Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
35 40 45
Gly Glu Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
50 55 60
Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
65 70 75 80
Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
85 90 95
Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
100 105 110
Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
115 120 125
Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
130 135 140
Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
145 150 155 160
Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
165 170 175
Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
180 185 190
Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
195 200 205
Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val
210 215 220
Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val
225 230 235 240
Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile
245 250 255
Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val
260 265 270
Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser
275 280 285
Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu
290 295 300
Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala
305 310 315 320
Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro
325 330 335

-continued

Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys
 340 345 350
 Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr
 355 360 365
 Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr
 370 375 380
 Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu
 385 390 395 400
 Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser
 405 410 415
 Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser
 420 425 430
 His Ser Pro Gly Lys
 435

<210> SEQ ID NO 18
 <211> LENGTH: 1311
 <212> TYPE: DNA
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Murine heavy B, variant sequence

<400> SEQUENCE: 18

gaggtcaaac tgctggagag tggaggggga ctggtgcagc caggcgggtc actgaagctg 60
 agctgcgccc cttccgctt cgacttttcc cggtactgga tgaattgggt gagacaggct 120
 cccggaaaag gcctggagtg gatcgggctg attaatcctg atagctccac catcaactac 180
 acaccaagtc tgaaggacaa attcattcatt tcacgcgata acgcaaagaa tactctgtat 240
 ctgcagatgt ctaaagtgcg aagtgaggac accgcactgt actattgtgc aagacagatg 300
 ggatactggg gacagggaac cacactgacc gtgtctagt ctaagactac cctcccagc 360
 gtgtatectc tggcacctgg ctccgcagca cagaccaatt ctatgggtgac actgggctgt 420
 ctggtcaagg ggtacttccc tgagccagtc acagtgactt ggaacagcgg cagcctgtca 480
 agcggcgtgc acacctttcc tgccgctctg cagagcgatc tgtatacact gtcctctagt 540
 gtcactgtgc cctcaagcac ctggccttcc gagaccgtga catgcaacgt cgcccatcct 600
 gcttcctcta caaaggtgga caagaaaatc gtcccacgag attgcggtctg taaaccatgc 660
 atttgactct tcccgaagt gagttcagtc ttcattcttc cacccaagcc aaaagcgtg 720
 ctgactatta cctgacacc caaggtcaca tgcgtggctg tggatatcag caaagacgat 780
 cccgaggtgc agttctcctg gttgtcgcac gatgtcgaag tgcacacagc ccagactcag 840
 cccagggagg aacagttcaa ttctacctt cgctctgtga gtgagctgcc tattatgcat 900
 caggactggc tgaatgaaa ggaattcaaa tgcagagtga actctgtctg atttcccgt 960
 cctatcgaga agactattag caagaccaa ggccagccta aagccccaca ggtgtacaca 1020
 atccctccac ccaaggaaca gatggctaag gataaagtga gcctgacatg tatgatcact 1080
 gacttctttc cegaggatat taccgtggaa tggcagtgga acgggcagcc cgcagagaac 1140
 tataagaata cacagcctat tatggacact gatggatcat acttcgtgta tagcaagctg 1200
 aacgtccaga aatctaattg ggaagccggc aacactttta cctgtagtgt gctgcatgaa 1260
 ggactgcata accatcatac tgaaaaagtc ctgtctcatt caccaggcaa a 1311

<210> SEQ ID NO 19
 <211> LENGTH: 437

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<212> TYPE: PRT
 <213> ORGANISM: artificial
 <220> FEATURE:
 <223> OTHER INFORMATION: Murine heavy B amino acid sequence

 <400> SEQUENCE: 19

 Glu Val Lys Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1 5 10 15

 Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Asp Phe Ser Arg Tyr
 20 25 30

 Trp Met Asn Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Ile
 35 40 45

 Gly Arg Ile Asn Pro Asp Ser Ser Thr Ile Asn Tyr Thr Pro Ser Leu
 50 55 60

 Lys Asp Lys Phe Ile Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr
 65 70 75 80

 Leu Gln Met Ser Lys Val Arg Ser Glu Asp Thr Ala Leu Tyr Tyr Cys
 85 90 95

 Ala Arg Gln Met Gly Tyr Trp Gly Gln Gly Thr Thr Leu Thr Val Ser
 100 105 110

 Ser Ala Lys Thr Thr Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser
 115 120 125

 Ala Ala Gln Thr Asn Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly
 130 135 140

 Tyr Phe Pro Glu Pro Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser
 145 150 155 160

 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr
 165 170 175

 Leu Ser Ser Ser Val Thr Val Pro Ser Ser Thr Trp Pro Ser Glu Thr
 180 185 190

 Val Thr Cys Asn Val Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys
 195 200 205

 Lys Ile Val Pro Arg Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val
 210 215 220

 Pro Glu Val Ser Ser Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val
 225 230 235 240

 Leu Thr Ile Thr Leu Thr Pro Lys Val Thr Cys Val Val Val Asp Ile
 245 250 255

 Ser Lys Asp Asp Pro Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val
 260 265 270

 Glu Val His Thr Ala Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser
 275 280 285

 Thr Phe Arg Ser Val Ser Glu Leu Pro Ile Met His Gln Asp Trp Leu
 290 295 300

 Asn Gly Lys Glu Phe Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala
 305 310 315 320

 Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro
 325 330 335

 Gln Val Tyr Thr Ile Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys
 340 345 350

 Val Ser Leu Thr Cys Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr
 355 360 365

 Val Glu Trp Gln Trp Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr
 370 375 380

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Gln Pro Ile Met Asp Thr Asp Gly Ser Tyr Phe Val Tyr Ser Lys Leu
385 390 395 400

Asn Val Gln Lys Ser Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser
405 410 415

Val Leu His Glu Gly Leu His Asn His His Thr Glu Lys Ser Leu Ser
420 425 430

His Ser Pro Gly Lys
435

<210> SEQ ID NO 20

<211> LENGTH: 642

<212> TYPE: DNA

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Murine light_Opt length: 725bp

<400> SEQUENCE: 20

gatattcaga tgactcagac tactttctcc ctgtctgcaa gtctggggga ccgagtgaca 60
atctcatgca ggcctccca gggaaatttc aactacctga attggttcca gcagaagcct 120
gatggcacag tgaactgct gatctactat actagctccc tgcacagtgg ggtcccatca 180
agattttctg gaagtggctc agggaccgac tatagcctga caatctccaa cctggagcca 240
gaagatattg ccacttacta ttgccagcag taccggaagc tgcctatac tttcggcggg 300
ggaaccaagc tggagatcaa aagagctgac gccgtccca cctgagcat ttttcccct 360
tctagtgaac agctgacctc tggcggggca agtgtggtct gtttctgaa caacttttac 420
cctaaagaca tcaacgtgaa gtggaaaatt gatggaagcg agaggcagaa cggcgtcctg 480
aattcctgga ccgaccagga tagcaaggac tccacatatt ctatgtcaag caccctgaca 540
ctgactaaag atgagtacga acgccataat agctatacat gtgaagctac tcataagacc 600
tctacctctc ctattgtgaa atcttttaac cgaatgaat gt 642

<210> SEQ ID NO 21

<211> LENGTH: 214

<212> TYPE: PRT

<213> ORGANISM: artificial

<220> FEATURE:

<223> OTHER INFORMATION: Murine light_Opt amino acid sequence

<400> SEQUENCE: 21

Asp Ile Gln Met Thr Gln Thr Thr Ser Ser Leu Ser Ala Ser Leu Gly
1 5 10 15

Asp Arg Val Thr Ile Ser Cys Ser Ala Ser Gln Gly Ile Ser Asn Tyr
20 25 30

Leu Asn Trp Phe Gln Gln Lys Pro Asp Gly Thr Val Lys Leu Leu Ile
35 40 45

Tyr Tyr Thr Ser Ser Leu His Ser Gly Val Pro Ser Arg Phe Ser Gly
50 55 60

Ser Gly Ser Gly Thr Asp Tyr Ser Leu Thr Ile Ser Asn Leu Glu Pro
65 70 75 80

Glu Asp Ile Ala Thr Tyr Tyr Cys Gln Gln Tyr Arg Lys Leu Pro Tyr
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg Ala Asp Ala Ala
100 105 110

Pro Thr Val Ser Ile Phe Pro Pro Ser Ser Glu Gln Leu Thr Ser Gly
115 120 125

Gly Ala Ser Val Val Cys Phe Leu Asn Asn Phe Tyr Pro Lys Asp Ile

专利名称(译)	淀粉样β病的抗体治疗		
公开(公告)号	US9908933	公开(公告)日	2018-03-06
申请号	US14/910172	申请日	2014-08-05
申请(专利权)人(译)	ST.文森特的医学研究所的研究		
当前申请(专利权)人(译)	ST.文森特的医学研究所的研究		
[标]发明人	MILES LUKE ANTHONY PARKER MICHAEL WILLIAM NERO TRACY LEAH		
发明人	MILES, LUKE ANTHONY PARKER, MICHAEL WILLIAM NERO, TRACY LEAH		
IPC分类号	C07K16/18 A61K39/395 G01N33/53 G01N33/68 C07H21/04		
CPC分类号	C07K16/18 G01N33/689 G01N33/6896 C07K2299/00 C07K2317/24 C07K2317/33 G01N2800/368 C07K2317/55 C07K2317/565 C07K2317/76 G01N2333/4709 G01N2800/2821 C07K2317/34 A61P25/28		
优先权	2013902922 2013-08-05 AU		
其他公开文献	US20160194385A1		
外部链接	Espacenet USPTO		

摘要(译)

本公开一般涉及治疗或预防与淀粉样蛋白β (Aβ) (包括阿尔茨海默氏病) 的病理形式相关的疾病或病症的方案。本文中使用的的是用于治疗 and 预防Aβ相关病理状况的试剂。

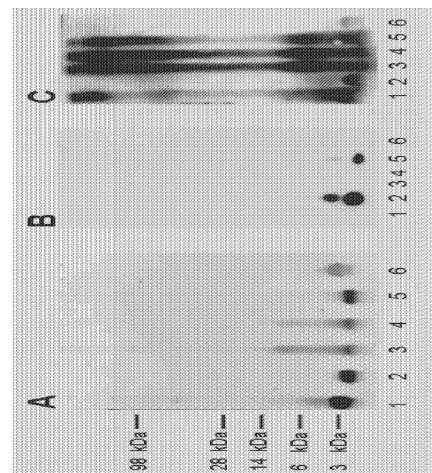


Figure 1