

(12) STANDARD PATENT
(19) AUSTRALIAN PATENT OFFICE

(11) Application No. **AU 2006306553 B9**

(54) Title
Anti-ADDL monoclonal antibody and use thereof

(51) International Patent Classification(s)
A61K 39/395 (2006.01)

(21) Application No: **2006306553** (22) Date of Filing: **2006.10.17**

(87) WIPO No: **WO07/050359**

(30) Priority Data

(31) Number (32) Date (33) Country
PCT/US2005/038125 **2005.10.21** **US**

(43) Publication Date: **2007.05.03**

(44) Accepted Journal Date: **2011.08.25**

(48) Corrigenda Journal Date: **2011.09.29**

(71) Applicant(s)
Merck Sharp & Dohme Corp.

(72) Inventor(s)
Kinney, Gene; Strohl, William R.; An, Zhiqiang

(74) Agent / Attorney
Freehills Patent & Trade Mark Attorneys, Level 43 101 Collins Street, Melbourne, VIC, 3000

(56) Related Art
WO1998/044955
US5750349

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

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
3 May 2007 (03.05.2007)

PCT

(10) International Publication Number
WO 2007/050359 A3

(51) International Patent Classification:
A61K 39/395 (2006.01)

(21) International Application Number:
PCT/US2006/040508

(22) International Filing Date: 17 October 2006 (17.10.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
PCT/US2005/381125
21 October 2005 (21.10.2005) US

(71) Applicant (for all designated States except US): **MERCK & CO., INC.** [US/US]; 126 E. Lincoln Avenue, Rahway, NJ 07065-0907 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **KINNEY, Gene** [US/US]; 4125 Justin Court, Collegeville, PA 19426 (US). **STROHL, William, R.** [US/US]; 1086 Tullo Farm Road, Bridgewater, NJ 08807 (US). **AN, Zhiqiang** [US/US]; 315 Gentry Lane, Ambler, PA 19002 (US).

(74) Agents: **LICATA, Jane, Massey et al.**; **LICATA & TYRRELL P.C.**, 66 E. Main Street, Marlton, NJ 08053 (US).

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

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments

(88) Date of publication of the international search report:
21 June 2007

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF

(57) Abstract: The present invention relates to antibodies that -differentially -recognize- -multi- dimensional conformations- of- β - derived diffusible ligands, also known as ADDLs. The antibodies of the invention can distinguish between Alzheimer's Disease and control human brain extracts and are useful in methods of detecting ADDLs and diagnosing Alzheimer's Disease. The present antibodies also block binding of ADDLs to neurons, assembly of ADDLs, and tau phosphorylation and are there useful in methods for the preventing and treating diseases associated with soluble oligomers of amyloid β 1-42.



WO 2007/050359 A3

- 1 -

ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF**10 Introduction**

This application is a continuation-in-part of PCT Application No. PCT/US2005/0038125 filed October 21, 2005.

Background of the Invention

15 Alzheimer's Disease is a progressive and degenerative dementia (Terry, et al. (1991) *Ann. Neurol.* 30:572-580; Coyle (1987) In: *Encyclopedia of Neuroscience*, Adelman (ed.), Birkhäuser, Boston-Basel-Stuttgart, pp 29-31,). In its early stages, Alzheimer's Disease manifests primarily
20 as a profound inability to form new memories (Selkoe (2002) *Science* 298:789-791), reportedly due to neurotoxins derived from amyloid beta (A β). A β is an amphipathic peptide whose abundance is increased by mutations and risk factors linked to Alzheimer's Disease. Fibrils formed from A β constitute
25 the core of amyloid plaques, which are hallmarks of an Alzheimer's Disease brain. Analogous fibrils generated *in vitro* are lethal to cultured brain neurons. These findings indicate that memory loss is a consequence of neuron death caused by fibrillar A β .

30 Despite strong experimental support for fibrillar A β and memory loss, a poor correlation exists between dementia and amyloid plaque burden (Katzman (1988) *Ann. Neurol.* 23:138-144). Moreover, transgenic hAPP mice (Dodart, et al. (2002) *Nat. Neurosci.* 5:452-457; Kotilinek, et al. (2002) *J. Neurosci.* 22:6331-6335), which develop age-dependent

- 2 -

amyloid plaques and, most importantly, age-dependent memory dysfunction, show that within 24 hours of vaccination with monoclonal antibodies against A β memory loss can be reversed with no change in plaque levels. Such findings are not consistent with a mechanism for memory loss dependent on neuron death caused by amyloid fibrils.

Additional neurologically active molecules formed by A β self-assembly have been suggested. These molecules include soluble A β oligomers, also referred to as A β -derived diffusible ligands or ADDLs. Oligomers are metastable and form at low concentrations of A β 1-42 (Lambert, et al. (1998) *Proc. Natl. Acad. Sci. USA* 95:6448-6453). A β oligomers rapidly inhibit long-term potentiation (LTP), a classic experimental paradigm for memory and synaptic plasticity. As such, memory loss stems from synapse failure, prior to neuron death and synapse failure by A β oligomers, not fibrils (Hardy & Selkoe (2002) *Science* 297:353-356). Soluble oligomers have been found in brain tissue and are strikingly elevated in Alzheimer's Disease (Kayed, et al. (2003) *Science* 300:486-489; Gong, et al. (2003) *Proc. Natl. Acad. Sci. USA* 100:10417-10422) and in hAPP transgenic mice Alzheimer's Disease models (Kotilinek, et al. (2002) *J. Neurosci.* 22:6331-6335; Chang, et al. (2003) *J. Mol. Neurosci.* 20:305-313).

A variety of Alzheimer's Disease treatment options have been suggested. Vaccine clinical trials have revealed that persons mounting a vigorous immune response to the vaccine exhibit cognitive benefit (Hock, et al. (2003) *Neuron* 38:547-554); however, frequency of CNS inflammation caused early termination of part of the trial (Birmingham & Frantz (2002) *Nat. Med.* 8:199-200). As an alternative to a vaccine, therapeutic antibodies that target ADDLs without binding monomers or fibrils have been suggested (Klein

- 3 -

(2002) *Neurochem. Int.* 41:345-352). ADDLs are highly antigenic, generating oligomer-selective polyclonal antibodies in rabbits at concentration of ~50 $\mu\text{g/mL}$ (Lambert, et al. (2001) *J. Neurochem.* 79:595-605). Results from transgenic mice models also suggest that antibodies can be successful in reversing memory decline (Dodart, et al. (2002) *Nat. Neurosci.* 5:452-457; U.S. Patent Application Serial No. 11/194,989). Accordingly, there is a need in the art for ADDL-selective therapeutic antibodies for the prevention and treatment of Alzheimer's Disease. The present invention meets this need.

Summary of the Invention

The present invention is an isolated antibody, or fragment thereof, capable of differentially recognizing a multi-dimensional conformation of one or more A β -derived diffusible ligands. In particular, the antibody of the instant invention has a complementary determining region (CDR) of Arg-Xaa₁-Leu-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa₁ is Gln or Ala; Xaa₂ is Ser or Gly; Xaa₃ is Pro, Ala, Lys, Arg, or Thr; Xaa₄ is Lys or Arg; Xaa₅ is Gly, Ser, or Lys; Xaa₆ is Val, Thr, Ile or Arg. In particular embodiments, the antibody of the present invention is in admixture with a pharmaceutically acceptable carrier. In other embodiments, the antibody of the present invention is in a kit. Still other embodiments embrace an antibody having heavy and light chain variable region sequences as set forth in SEQ ID NO:108 and SEQ ID NO:112. An antibody having heavy and light chain sequences as set forth in SEQ ID NO:138 and SEQ ID NO:140 is also provided.

Methods for preventing binding of A β -derived diffusible ligands to a neuron and inhibiting assembly of

- 4 -

A β -derived diffusible ligands employing an antibody or antibody fragment which binds a multi-dimensional conformation of one or more A β -derived-diffusible ligands are also provided.

5 The present invention further embraces a method for prophylactically or therapeutically treating a disease associated with A β -derived diffusible ligands using an antibody of the instant invention. Administration of an antibody of the invention can prevent binding of A β -derived
10 diffusible ligands to a neuron thereby preventing or treating the disease associated with A β -derived diffusible ligands.

 The present invention is also a method for identifying a therapeutic agent that prevents the binding of A β -derived
15 diffusible ligands to a neuron. This method of the invention involves contacting a neuron with A β -derived diffusible ligands in the presence of an agent and using an antibody of the present invention to determine binding of the A β -derived diffusible ligands to the neuron in the
20 presence of the agent.

 The present invention also embraces a method for detecting A β -derived diffusible ligands in a sample and a method for diagnosing a disease associated with A β -derived diffusible ligands. Such methods involve contacting a
25 sample with an antibody of the instant invention so that the A β -derived diffusible ligands can be detected and a disease associated with A β -derived diffusible ligands can be diagnosed.

30 **Brief Description of the Drawings**

 Figure 1 shows the nucleic acid sequences for the heavy (Figure 1A) and light (Figure 1B) chain variable regions for murine anti-ADDL antibody 20C2. Lower case

- 5 -

letters indicate the antibody leader sequences and uppercase letters indicate antibody variable region sequences. The nucleotides coding for the complementary determining regions (CDRs) are underlined.

5 Figure 2 shows comparisons of heavy (Figure 2A) and light (Figure 2B) chain variable region amino acid sequences of murine antibody 20C2 and humanized antibodies, Hu20C2 (CDR grafted) and Hu20C2A3 (veneered). Sequences are presented as comparisons between the 20C2 mouse sequence,
10 the most homologous human genomic sequence and the humanized sequences. Sequence differences in the frame regions between murine 20C2 and humanized Hu20C2A3 are in bold. Sequence differences in the underlined CDR regions between humanized Hu20C2A3 and murine 20C2 are in bold and
15 indicated with an *. CDRs are underlined.

 Figure 3 shows nucleic acid sequences for the heavy (Figures 3A and 3B) and light (Figure 3C) chain variable regions (HCVRs and LCVRs, respectively) for humanized anti-ADDL antibody Hu20C2 (CDR grafted). Two humanized versions
20 of the Hu20C2 heavy chain were generated (HCVRA and HCVRB) that differ by one amino acid at position 24. In Hu20C2 HCVRA the human amino acid was used and in Hu20C2 HCVRB the mouse amino acid was used. Variable region sequences were cloned into full heavy and light chain antibody expression
25 vectors.

 Figure 4 shows the annotated amino acid sequences and nucleotide sequences of Hu20C2 humanized antibody in Fab phage-display vector pFab4. Amino acid sequence for heavy chain version A (Figure 4A), heavy chain version B (Figure
30 4B), and the light chain (Figure 4C) of Hu20C2 humanized antibody in Fab phage-display vector pFab4 are in italic and underlined regions are as indicated. Nucleotide sequence of heavy chain version A fused with the light

- 6 -

chain of Hu20C2 in pFab4 vector is shown in Figure 4D-4E with sequences encoding the Hu20C2 antibody sequences shown in lowercase.

Figure 5 depicts the design and primers employed in preparing two light chain CDR3 libraries, namely LC3-1 and LC3-2 (Figure 5A), and three heavy chain CDR3 libraries, namely 20C2B-39HC₃-1, 20C2B-39HC₃-2, and 20C2B-39HC₃-3 (Figure 5B), for respectively generating affinity matured Hu20C2 light and heavy chain CDR3s. Restriction endonuclease recognition sites used for cloning are indicated in *italic*. Uppercase indicates nucleic acids encoding antibody variable region sequences. Nucleic acids encoding CDRs are underlined. Biotin-labeled primers are indicated.

Figure 6 shows a comparison of the amino acid sequence of human antibody constant regions and the sequence of IgG2m4. The asterisk indicates a glycosylation site at Asn297. Regions of FcRn binding are indicated. Sequences in which IgG2m4 is different from IgG2 are underlined.

Figure 7 shows the amino acid (Figures 7A and 7C) and nucleotide (Figures 7B and 7D) sequences for the full IgG2m4 humanized heavy chain (Figures 7A and 7B) and humanized Kappa light chain (Figures 7C and 7D) for anti-ADDL antibody Hu20C2A3. Underlining indicates variable region sequences. The remaining sequences are constant region sequences.

Figure 8 shows interactions between A β 40 monomer or ADDLs with Hu20C2A3 produced by two different systems, CHO (Figure 8A) or *Pichia* (Figure 8B), as determined by ELISA.

Figure 9 shows Hu20C2A3 inhibition of bADDL binding to primary hippocampal neurons.

Figure 10 shows fluorescent thermal melt analysis of Hu20C2A3.

- 7 -

Figure 11 shows plasma A β x-40 levels (pM) of APP-YAC mice following intravenous injection of Hu20C2A3, irrelevant control or vehicle. Hu20C2A3 was prepared by stable transfection of CHO cells or *Pichia*. A β x-40 was
5 determined using a 4G8/G2-10 ELISA. ***, $p < 0.001$ by Tukey-Kramer HSD post-hoc testing. Error bars = SEM; N=6/group.

Detailed Description of the Invention

Monoclonal antibodies, which differentially recognize
10 multi-dimensional conformations of A β -derived diffusible ligands (*i.e.*, ADDLs), have now been generated. Antibodies of this invention are derived from the murine monoclonal antibody 20C2. Murine 20C2 is known in the art for exhibiting the following characteristics. Murine 20C2 is an
15 IgG1 antibody which binds to both synthetic and endogenous ADDLs bound to cultured hippocampal cells. Furthermore, this antibody can block both endogenous and synthetic ADDL binding to cultured cells, abate the binding of biotinylated ADDLs (bADDLs) to neurons, and prevent tau
20 phosphorylation. The core linear epitope for 20C2 is Glu-Phe-Arg-His-Asp-Ser (SEQ ID NO:1), corresponding to amino acid residues 3-8 of A β 1-42, with a conformational epitope that is dependent upon elements from within residues 17-42 of A β , but only when assembled.

25 The instant antibodies are humanized and, in some embodiments affinity-matured derivatives of murine 20C2. Like the murine 20C2 antibody, the antibodies disclosed herein exhibit a high degree of selectivity for multi-dimensional conformations of ADDLs, with minimal detection
30 of monomer A β peptides. Advantageously, the instant antibodies identify endogenous oligomers in Alzheimer's Disease brain slices and inhibit binding of bADDLs to neurons. Moreover, the instant antibodies provide a

- 8 -

significant and robust increase in plasma A β x-40 levels, an increase in which is known to be associated with an ultimate lowering of brain A β . Accordingly, the antibodies of this invention find use in the prevention of ADDL binding to neurons and assembly of ADDLs and the treatment of ADDL-related diseases including Alzheimer's Disease.

Accordingly, the present invention is an isolated antibody that differentially recognizes one or more multi-dimensional conformations of ADDLs. An antibody of the instant invention is said to be isolated when it is present in the substantial absence of other biological macromolecules of the same type. Thus, an "isolated antibody" refers to an antibody which is substantially free of other antibodies; however, the molecule may include some additional agents or moieties which do not deleteriously affect the basic characteristics of the antibody (e.g., binding specificity, neutralizing activity, etc.).

An antibody which is capable of specifically binding one or more multi-dimensional conformations of ADDLs, binds particular ADDLs derived from the oligomerization of A β 1-42, but like murine 20C2 does not cross-react with other A β peptides, namely A β 1-12, A β 1-28, A β 1-40, and A β 12-28 as determined by western blot analyses; and preferentially bind ADDLs in solution. Specific binding between two entities generally refers to an affinity of at least 10^6 , 10^7 , 10^8 , 10^9 , or 10^{10} M⁻¹. Affinities greater than 10^8 M⁻¹ are desired to achieve specific binding.

In particular embodiments, an antibody that is capable of specifically binding a multi-dimensional conformation of one or more ADDLs is also raised against (i.e., an animal is immunized with) multi-dimensional conformations of ADDLs. In other embodiments, an antibody that is capable of specifically binding a multi-dimensional conformation of

- 9 -

one or more ADDLs is raised against a low n-mer-forming peptide such as A β 1-42[Nle35-Dpro37].

The term "epitope" refers to a site on an antigen to which B and/or T cells respond or a site on a molecule against which an antibody will be produced and/or to which an antibody will bind. For example, an epitope can be recognized by an antibody defining the epitope.

A linear epitope is an epitope wherein an amino acid primary sequence comprises the epitope recognized. A linear epitope typically includes at least 3, and more usually, at least 5, for example, about 6 to about 10 amino acids in a unique sequence.

A conformational epitope, in contrast to a linear epitope, is an epitope wherein the primary sequence of the amino acids comprising the epitope is not the sole defining component of the epitope recognized (e.g., an epitope wherein the primary sequence of amino acids is not necessarily recognized by the antibody defining the epitope). Typically a conformational epitope encompasses an increased number of amino acids relative to a linear epitope. With regard to recognition of conformational epitopes, the antibody recognizes a three-dimensional structure of the peptide or protein. For example, when a protein molecule folds to form a three-dimensional structure, certain amino acids and/or the polypeptide backbone forming the conformational epitope become juxtaposed enabling the antibody to recognize the epitope. Methods of determining conformation of epitopes include but are not limited to, for example, x-ray crystallography, two-dimensional nuclear magnetic resonance spectroscopy and site-directed spin labeling and electron paramagnetic resonance spectroscopy. See, for example, Epitope Mapping

- 10 -

Protocols in Methods in Molecular Biology (1996) Vol. 66, Morris (Ed.).

A β -derived diffusible ligands -- or -- ADDLs -- refer -- to -- soluble oligomers of amyloid β 1-42 which are desirably composed of aggregates of less than eight or nine amyloid β 1-42 peptides and are found associated with Alzheimer's Disease. This is in contrast to high molecular weight aggregation intermediates, which form strings of micelles leading to fibril formation.

As exemplified herein, the instant antibody binds or recognizes at least one multi-dimensional conformation of an ADDL. In particular embodiments, the instant antibody binds at least two, at least three, or at least four multi-dimensional conformations of an ADDL. Multi-dimensional conformations of ADDLs are intended to encompass dimers, trimers, tetramers pentamers, hexamers, heptamers, octamers, nonamers, decamers, etc. as defined by analysis via SDS-PAGE. Because trimer, tetramer, etc. designations can vary with the assay method employed (see, e.g., Bitan, et al. (2005) *Amyloid* 12:88-95) the definition of trimer, tetramer, and the like, as used herein, is according to SDS-PAGE analysis. As such, the antibody of the instant invention has oligomer-specific characteristics. In particular embodiments, a multi-dimensional conformation of an ADDL is associated with a specific polypeptide structure which results in a conformational epitope that is recognized by an antibody of the present invention. In other embodiments, an antibody of the invention specifically binds a multi-dimensional conformation ADDL having a size range of approximately a trimer or tetramer, which have molecular weights in excess of >50 kDa.

In certain embodiments, in addition to binding to a multi-dimensional conformation, the instant antibody binds

- 11 -

to a selected linear epitope of amyloid β 1-42. A linear epitope of an ADDLs is intended as a four, five, six or more amino acid residue peptide located in the N-terminal 10, 11, 12, 15 or 20 amino acid residues of amyloid β 1-42. In particular embodiments, an antibody of the invention specifically binds to a linear epitope within residues 1-10, 1-8, 3-10, or 3-8 of amyloid β 1-42. An exemplary linear epitope of amyloid β 1-42 which is bound by a humanized antibody of the invention is amino acid residues Glu-Phe-Arg-His-Asp-Ser (SEQ ID NO:1).

While antibodies of the instant invention may have similar linear epitopes, such linear epitopes are not wholly indicative of the binding characteristics of the instant antibodies (*i.e.*, ability to block ADDL binding to neurons, prevent tau phosphorylation and inhibit ADDL assembly) because, as is well-known to the skilled artisan, the linear epitope may only correspond to a portion of the antigen's epitope (see, *e.g.*, Breitling and Dübel (1999) *In: Recombinant Antibodies*, John Wiley & Sons, Inc., NY, pg. 115). For example, murine 20C2 is known to bind assemblies of charge-inverted, truncated A β 7-42 peptide, which lack the linear epitope for 20C2 (*i.e.*, amino acid residues 3-8) and contain a very different sequence corresponding to residues 7-16 of A β . Therefore, 20C2, as well as humanized derivatives thereof, bind to conformational epitopes that depend upon elements from within residues 17-42 of A β , but only when in a multidimensional conformation. The antibody of the instant invention can be distinguished from those of the art as being capable of differentially recognizing multidimensional ADDLs and accordingly differentially blocking ADDL binding to neurons, differentially preventing tau

- 12 -

phosphorylation and differentially inhibiting ADDL assembly.

An antibody, as used in accordance with the instant invention includes, but is not be limited to, monoclonal antibodies, and chimeric, human (e.g. isolated from B cells), humanized, neutralizing, bispecific or single chain antibodies thereof. In one embodiment, an antibody of the instant invention is monoclonal. For the production of antibodies, various hosts including goats, rabbits, chickens, rats, mice, humans, and others, can be immunized by injection with synthetic or natural ADDLs. Methods for producing antibodies are well-known in the art. See, e.g., Kohler and Milstein ((1975) *Nature* 256:495-497) and Harlow and Lane (*Antibodies: A Laboratory Manual* (Cold Spring Harbor Laboratory, New York (1988))).

Depending on the host species, various adjuvants can be used to increase the immunological response. Adjuvants used in accordance with the instant invention desirably augment the intrinsic response to ADDLs without causing conformational changes in the immunogen that affect the qualitative form of the response. Particularly suitable adjuvants include 3 De-O-acylated monophosphoryl lipid A (MPL™; RIBI ImmunoChem Research Inc., Hamilton, MT; see GB 2220211) and oil-in-water emulsions, such as squalene or peanut oil, optionally in combination with immune stimulants, such as monophosphoryl lipid A (see Stoute, et al. (1997) *N. Engl. J. Med.* 336:86-91), muramyl peptides (e.g., N-acetylmuramyl-L-threonyl-D-isoglutamine (thr-MDP), N-acetyl-normuramyl-L-alanyl-D-isoglutamine (nor-MDP), N-acetylmuramyl-L-alanyl-D-isoglutaminyl-L-alanine-2-(1'-2'dipalmitoyl-sn-glycero-3-hydroxyphosphoryloxy)-ethylamine (E-PE), N-acetylglucosaminyl-N-acetylmuramyl-L-Al-D-isoglu-L-Ala-dipalmitoxy propylamide (DTP-DPP)), or other

- 13 -

bacterial cell wall components. Specific examples of oil-in-water emulsions include MF59 (WO 90/14837), containing 5% Squalene, 0.5% TWEEN™ 80, and 0.5% SPAN- 85 (optionally containing various amounts of MTP-PE) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA); SAF containing 10% Squalene, 0.4% TWEEN™ 80, 5% PLURONIC®-blocked polymer L121, and thr-MDP, either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion; and RIBI™ adjuvant system (RAS) (Ribi ImmunoChem, Hamilton, MT) containing 2% squalene, 0.2% TWEEN™ 80, and one or more bacterial cell wall components such as monophosphoryllipid A, trehalose dimycolate (TDM), and cell wall skeleton (CWS).

Another class of adjuvants is saponin adjuvants, including ISCOMs (immunostimulating complexes) and ISCOMATRIX® (CSL Ltd., Parkville, Australia). Other suitable adjuvants include Complete Freund's Adjuvant (CFA), Incomplete Freund's Adjuvant (IFA), mineral gels such as aluminum hydroxide, and surface-active substances such as lysolecithin, PLURONIC® polyols, polyanions, peptides, CpG (WO 98/40100), keyhole limpet hemocyanin, dinitrophenol, and cytokines such as interleukins (IL-1, IL-2, and IL-12), macrophage colony stimulating factor (M-CSF), and tumor necrosis factor (TNF). Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are particularly suitable.

An antibody to a multi-dimensional conformation ADDL is generated by immunizing an animal with ADDLs. Generally, ADDLs can be generated synthetically or by recombinant fragment expression and purification. Synthetic ADDLs can be prepared as disclosed herein or in accordance with the methods disclosed in U.S. Patent No. 6,218,506 or in co-

- 14 -

pending applications US Serial Nos. 60/621,776, 60/652,538, 60/695,528 and 60/695,526. Further, ADDLs can be fused with another protein such as keyhole limpet hemocyanin to generate an antibody against the chimeric molecule. The ADDLs can be conformationally constrained to form an epitope useful as described herein and furthermore can be associated with a surface for example, physically attached or chemically bonded to a surface in such a manner so as to allow for the production of a conformation which is recognized by the antibodies of the present invention.

Monoclonal antibodies to multi-dimensional conformations of ADDLs can be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique (Kohler, et al. (1975) *Nature* 256:495-497; Kozbor, et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; Cole, et al. (1984) *Mol. Cell Biol.* 62:109-120).

In particular embodiments, the instant antibodies are humanized. Humanized or chimeric antibodies can be produced by splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity (see Morrison, et al. (1984) *Proc. Natl. Acad. Sci.* 81, 6851-6855; Neuberger, et al. (1984) *Nature* 312:604-608; Takeda, et al. (1985) *Nature* 314:452-454; Queen, et al. (1989) *Proc. Natl. Acad. Sci. USA* 86:10029-10033; WO 90/07861). For example, a mouse antibody is expressed as the Fv or Fab fragment in a phage selection vector. The gene for the light chain (and in a parallel experiment, the gene for the heavy chain) is exchanged for a library of human antibody genes. Phage antibodies, which

- 15 -

still bind the antigen, are then identified. This method, commonly known as chain shuffling, provided humanized antibodies that should bind the same epitope as the mouse antibody from which it descends (Jespers, et al. (1994) *Biotechnology* NY 12:899-903). As an alternative, chain shuffling can be performed at the protein level (see, Figini, et al. (1994) *J. Mol. Biol.* 239:68-78).

Human antibodies can also be obtained using phage-display methods. See, e.g., WO 91/17271 and WO 92/01047. In these methods, libraries of phage are produced in which members display different antibodies on their outer surfaces. Antibodies are usually displayed as Fv or Fab fragments. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to ADDLs. Human antibodies against ADDLs can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus. See, e.g., WO 93/12227 and WO 91/10741. Human antibodies can be selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody. Such antibodies generally retain the useful functional properties of the mouse antibodies. Human polyclonal antibodies can also be provided in the form of serum from humans immunized with an immunogenic agent. Optionally, such polyclonal antibodies can be concentrated by affinity purification using ADDLs as an affinity reagent.

As exemplified herein, humanized antibodies can also be produced by veneering or resurfacing of murine antibodies. Veneering involves replacing only the surface fixed region amino acids in the mouse heavy and light variable regions with those of a homologous human antibody

- 16 -

sequence. Replacing mouse surface amino acids with human residues in the same position from a homologous human sequence has been shown to reduce the immunogenicity of the mouse antibody while preserving its ligand binding. The replacement of exterior residues generally has little, or no, effect on the interior domains, or on the interdomain contacts. (See, e.g., U.S. Patent No. 6,797,492).

Human or humanized antibodies can be designed to have IgG, IgD, IgA, IgM or IgE constant regions, and any isotype, including IgG1, IgG2, IgG3 and IgG4. In particular embodiments, an antibody of the invention is IgG or IgM, or a combination thereof. Other embodiments of the present invention embrace a constant region formed by selective incorporation of human IgG4 sequences into a standard human IgG2 constant region. An exemplary mutant IgG2 Fc is IgG2m4, set forth herein as SEQ ID NO:140. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains and light chains or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer. Techniques for the production of single chain antibodies are well-known in the art.

Exemplary humanized antibodies derivatives of murine 20C2 monoclonal antibody are provided herein by CDR grafting and veneering. Amino acid sequences for IgG2M4 heavy chain variable regions, as well as kappa light chain variable regions for humanized 20C2 (i.e., Hu20C2A3) generated by veneering are presented in Figures 7A and 7C and set forth herein as SEQ ID NO:141 and SEQ ID NO:143.

Diabodies are also contemplated. A diabody refers to an engineered antibody construct prepared by isolating the binding domains (both heavy and light chain) of a binding antibody, and supplying a linking moiety which joins or

- 17 -

operably links the heavy and light chains on the same polypeptide chain thereby preserving the binding function (see, Holliger et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6444; Poljak (1994) *Structure* 2:1121-1123). This forms, in essence, a radically abbreviated antibody, having only the variable domain necessary for binding the antigen. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. These dimeric antibody fragments, or diabodies, are bivalent and bispecific. The skilled artisan will appreciate that any method to generate diabodies can be used. Suitable methods are described by Holliger, et al. (1993) *supra*, Poljak (1994) *supra*, Zhu, et al. (1996) *Biotechnology* 14:192-196, and U.S. Patent No. 6,492,123, incorporated herein by reference.

Fragments of an isolated antibody of the invention are also expressly encompassed by the instant invention. Fragments are intended to include Fab fragments, $F(ab')_2$ fragments, $F(ab')$ fragments, bispecific scFv fragments, Fd fragments and fragments produced by a Fab expression library, as well as peptide aptamers. For example, $F(ab')_2$ fragments are produced by pepsin digestion of the antibody molecule of the invention, whereas Fab fragments are generated by reducing the disulfide bridges of the $F(ab')_2$ fragments. Alternatively, Fab expression libraries can be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (see Huse, et al. (1989) *Science* 254:1275-1281). In particular embodiments, antibody fragments of the present invention are fragments of neutralizing antibodies which retain the variable region binding site thereof. Exemplary are $F(ab')_2$ fragments, $F(ab')$ fragments, and Fab fragments. See

- 18 -

generally Immunology: Basic Processes (1985) 2nd edition, J. Bellanti (Ed.) pp. 95-97.

Peptide aptamers which differentially recognize multi-dimensional conformations of ADDLs can be rationally designed or screened for in a library of aptamers (e.g., provided by Aptanomics SA, Lyon, France). In general, peptide aptamers are synthetic recognition molecules whose design is based on the structure of antibodies. Peptide aptamers consist of a variable peptide loop attached at both ends to a protein scaffold. This double structural constraint greatly increases the binding affinity of the peptide aptamer to levels comparable to that of an antibody (nanomolar range).

Exemplary nucleic acid sequences encoding heavy and light chain variable regions for use in producing antibody and antibody fragments of the instant invention are respectively disclosed herein in Figures 7B and 7D (i.e., SEQ ID NOs:142 and 144). As will be appreciated by the skilled artisan, the heavy chain variable regions disclosed herein can be used in combination with any one of the light chain variable regions disclosed herein to generate antibodies with modified affinities, dissociate constants, epitopes and the like.

Antibodies or antibody fragments of the present invention can have additional moieties attached thereto. For example, a microsphere or microparticle can be attached to the antibody or antibody fragment, as described in U.S. Patent No. 4,493,825, the disclosure of which is incorporated herein by reference.

Moreover, particular embodiment embrace antibody or antibody fragments which are mutated and selected for increased antigen affinity, neutralizing activity (i.e., the ability to block binding of ADDLs to neuronal cells or

- 19 -

the ability to block ADDL assembly), or a modified dissociation constant. Mutator strains of *E. coli* (Low, et al. (1996) *J. Mol. Biol.* 260:359-368), chain-shuffling (Figini, et al. (1994) *supra*), and PCR mutagenesis are established methods for mutating nucleic acid molecules encoding antibodies. By way of illustration, increased affinity can be selected for by contacting a large number of phage antibodies with a low amount of biotinylated antigen so that the antibodies compete for binding. In this case, the number of antigen molecules should exceed the number of phage antibodies, but the concentration of antigen should be somewhat below the dissociation constant. Thus, predominantly mutated phage antibodies with increased affinity bind to the biotinylated antigen, while the larger part of the weaker affinity phage antibodies remains unbound. Streptavidin can then assist in the enrichment of the higher affinity, mutated phage antibodies from the mixture (Schier, et al. (1996) *J. Mol. Biol.* 255:28-43). Exemplary affinity-maturated light chain CDR3 amino acid sequences are disclosed herein (see Tables 6 and 7), with particular embodiments embracing a light chain CDR3 amino acid sequence of Xaa₁-Gln-Xaa₂-Thr-Arg-Val-Pro-Leu-Thr (SEQ ID NO:2), wherein Xaa₁ is Phe or Leu, and Xaa₂ is Ala or Thr. Affinity-maturated heavy chain CDR3 amino acid sequences are also provided herein. An exemplary heavy chain CDR3 amino acid sequence is set forth herein as Arg-Gln-Leu-Gly-Thr-Arg-Gly-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:3). The present invention also embraces derivatives of this CDR3, e.g., Arg-Ala-Leu-Ser-Pro-Arg-Ser-Ile-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:4), Arg-Gln-Leu-Gly-Ala-Arg-Lys-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:5), Arg-Gln-Leu-Gly-Pro-Arg-Lys-Arg-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:6), Arg-Gln-Leu-Gly-Lys-Leu-Lys-Thr-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:7), or Arg-

- 20 -

Gln-Leu-Gly-Arg-Arg-Ser-Val-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:8), wherein differences with the Hu20C2A3 heavy chain CDR3 are underlined. In this regard, the present invention specifically embraces an anti-ADDL antibody having a CDR3 amino acid sequence of Arg-Xaa₁-Leu-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa₁ is Gln or Ala; Xaa₂ is Ser or Gly; Xaa₃ is Pro, Ala, Lys, Arg, or Thr; Xaa₄ is Lys or Arg; Xaa₅ is Gly, Ser, or Lys; Xaa₆ is Val, Thr, Ile or Arg

Other antibody derivatives encompassed within the scope of the present invention include any humanized antibody identical to Hu20C2A3's variable regions except with a one amino acid residue difference in the frame region of the light chain (e.g., Leu-Pro-Val-Thr-Pro-Gly-Glu-Pro-Ala-Ser, SEQ ID NO:10).

For some therapeutic applications it may be desirable to reduce the dissociation of the antibody from the antigen. To achieve this, phage antibodies are bound to biotinylated antigen and an excess of unbiotinylated antigen is added. After a period of time, predominantly the phage antibodies with the lower dissociation constant can be harvested with streptavidin (Hawkins, et al. (1992) *J. Mol. Biol.* 226:889-96).

Various immunoassays including those disclosed herein can be used for screening to identify antibodies, or fragments thereof, having the desired specificity for multi-dimensional conformations of ADDLs. Numerous protocols for competitive binding (e.g., ELISA), latex agglutination assays, immunoradiometric assays, kinetics (e.g., BIACORE™ analysis) using either polyclonal or monoclonal antibodies, or fragments thereof, are well-known in the art. Such immunoassays typically involve the measurement of complex formation between a specific

- 21 -

antibody and its cognate antigen. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes is suitable, but a competitive binding assay can also be employed. Such assays
5 can also be used in the detection of multi-dimensional conformations of ADDLs in a sample.

An antibody or antibody fragment can also be subjected to other biological activity assays, e.g., displacement of ADDL binding to neurons or cultured hippocampal cells or
10 blockade of ADDL assembly, in order to evaluate neutralizing or pharmacological activity and potential efficacy as a prophylactic or therapeutic agent. Such assays are described herein and are well-known in the art.

Antibodies and fragments of antibodies can be produced
15 and maintained as hybridomas or alternatively recombinantly produced in any well-established expression system including, but not limited to, *E. coli*, yeast (e.g., *Saccharomyces* spp. and *Pichia* spp.), baculovirus, mammalian cells (e.g., myeloma, CHO, COS), plants, or transgenic
20 animals (Breitling and Dübel (1999) In: Recombinant Antibodies, John Wiley & Sons, Inc., NY, pp. 119-132). Antibodies and fragments of antibodies can be isolated using any appropriate methods including, but not limited to, affinity chromatography, immunoglobulins-binding
25 molecules (e.g., proteins A, L, G or H), tags operatively linked to the antibody or antibody fragment (e.g., His-tag, FLAG®-tag, Strep tag, c-myc tag) and the like. See, Breitling and Dübel (1999) *supra*.

Antibodies and antibody fragments of the instant
30 invention have a variety of uses including, diagnosis of diseases associated with accumulation of ADDLs, blocking or inhibiting binding of ADDLs to neuronal cells, blocking ADDL assembly, prophylactically or therapeutically treating

- 22 -

a disease associated with ADDLs, identifying therapeutic agents that prevent binding of ADDLs to neurons, and preventing the phosphorylation of tau protein at Ser202/Thr205.

5 Antibody and antibody fragments of the instant invention are also useful in a method for blocking or inhibiting binding of ADDLs to neuronal cells. This method of the invention is carried out by contacting a neuron, *in vitro* or *in vivo*, with an antibody or antibody fragment of
10 the present invention so that binding of ADDLs to the neuron is blocked. In particular embodiments, an antibody or antibody fragment of the instant invention achieves at least a 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 97% decrease in the binding of ADDLs as compared to
15 binding of ADDLs in the absence of the antibody or antibody fragment. The degree to which an antibody can block the binding of ADDLs to a neuron can be determined in accordance with the methods disclosed herein, *i.e.*, immunocytochemistry or cell-based alkaline phosphatase
20 assay or any other suitable assay. Antibodies particularly useful for decreasing binding of ADDLs to neuronal cells include anti-ADDL antibodies having a CDR3 amino acid sequence set forth in SEQ ID NO:9, as well as derivatives and fragments thereof.

25 Antibody and antibody fragments of the instant invention are further useful in a method for blocking or inhibiting assembly of ADDLs. This method involves contacting a sample containing amyloid β 1-42 peptides with an antibody or antibody fragment of the instant invention
30 so that ADDL assembly is inhibited. The degree to which an antibody can block the assembly of ADDLs can be determined in accordance with the methods disclosed herein, *i.e.*, FRET or fluorescence polarization or any other suitable assay.

- 23 -

Antibodies particularly useful for blocking the assembly of ADDLs include anti-ADDL antibodies having a CDR3 amino acid sequence set forth in SEQ. ID. NO:9, as well as derivatives and fragments thereof.

5 Antibodies disclosed herein are also useful in methods for preventing the phosphorylation of tau protein at Ser202/Thr205. This method involves contacting a sample containing tau protein with an antibody or antibody fragment of the instant invention so that binding of ADDLs
10 to neurons is blocked thereby preventing phosphorylation of tau protein. The degree to which an antibody can prevent the phosphorylation of tau protein at Ser202/Thr205 can be determined in accordance with the methods disclosed herein or any other suitable assay.

15 Blocking or decreasing binding of ADDLs to neurons, inhibiting assembly of ADDLs, and preventing the phosphorylation of tau protein at Ser202/Thr205 all find application in methods of prophylactically or therapeutically treating a disease associated with the
20 accumulation of ADDLs. Accordingly, the present invention also embraces the use of an antibody or antibody fragment of the instant invention to prevent or treat a disease associated with the accumulation of ADDLs (e.g. Alzheimer's or similar memory-related disorders). Evidence in the art
25 indicates that elevated levels of A β , but not necessarily aggregated plaque, are causative for Alzheimer's Disease-associated dementia and subsequent tau abnormalities. A β -derived diffusible ligands are directly implicated in neurotoxicity associated with Alzheimer's Disease. The art
30 indicates that ADDLs are elevated in transgenic mice and Alzheimer's Disease patients and modulate functional activity associated with mnemonic processes in animal models. Thus, removing this form of A β could provide relief

- 24 -

from the neurotoxicity associated with Alzheimer's Disease. As such, treatment with the instant antibody, which reduces central nervous system ADDL load, could prove efficacious for the treatment of Alzheimer's Disease. Patients amenable to treatment include individuals at risk of disease but not exhibiting symptoms, as well as patients presently exhibiting symptoms. In the case of Alzheimer's Disease, virtually anyone is at risk of suffering from Alzheimer's Disease if he or she lives long enough. Therefore, the antibody or antibody fragments of the present invention can be administered prophylactically to the general population without the need for any assessment of the risk of the subject patient. The present methods are especially useful for individuals who have a known genetic risk of Alzheimer's Disease. Such individuals include those having relatives who have been diagnosed with the disease, and those whose risk is determined by analysis of genetic or biochemical markers. Genetic markers of risk for Alzheimer's Disease include mutations in the APP gene, particularly mutations at position 717 and positions 670 and 671 referred to as the Hardy and Swedish mutations respectively. Other markers of risk are mutations in the presenilin genes, PS1 and PS2, and ApoE4, family history of Alzheimer's Disease, hypercholesterolemia or atherosclerosis. Individuals presently suffering from Alzheimer's Disease can be recognized from characteristic dementia, as well as the presence of risk factors described above. In addition, a number of diagnostic tests are available for identifying individuals who have Alzheimer's Disease. These include measurement of CSF tau and A β 1-42 levels. Individuals suffering from Alzheimer's Disease can also be diagnosed by ADRDA criteria or the method disclosed herein.

- 25 -

In asymptomatic patients, treatment can begin at any age (e.g., 10, 20, 30 years of age). Usually, however, it is not necessary to begin treatment until a patient reaches 40, 50, 60 or 70 years of age. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying for the presence of ADDLs over time.

In therapeutic applications, a pharmaceutical composition or medicament containing an antibody or antibody fragment of the invention is administered to a patient suspected of, or already suffering from such a disease associated with the accumulation of ADDLs in an amount sufficient to cure, or at least partially arrest, the symptoms of the disease (biochemical, histologic and/or behavioral), including its complications and intermediate pathological phenotypes in development of the disease. In prophylactic applications, a pharmaceutical composition or medicament containing an antibody or antibody fragment of the invention is administered to a patient susceptible to, or otherwise at risk of, a disease associated with the accumulation of ADDLs in an amount sufficient to achieve passive immunity in the patient thereby eliminating or reducing the risk, lessening the severity, or delaying the outset of the disease, including biochemical, histologic and/or behavioral symptoms of the disease, its complications and intermediate pathological phenotypes presenting during development of the disease. In some methods, administration of agent reduces or eliminates myocognitive impairment in patients that have not yet developed characteristic Alzheimer's pathology. In particular embodiments, an effective amount of an antibody or antibody fragment of the invention is an amount which achieves at least a 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or 97% decrease in the binding of ADDLs to

- 26 -

neurons in the patient as compared to binding of ADDLs in the absence of treatment. As such, impairment of long-term potentiation/memory formation is decreased.

Effective doses of the compositions of the present invention, for the treatment of the above described conditions vary depending upon many different factors, including means of administration, physiological state of the patient, whether the patient is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic. Usually, the patient is a human but nonhuman mammals such as dogs or transgenic mammals can also be treated.

Treatment dosages are generally titrated to optimize safety and efficacy. For passive immunization with an antibody or antibody fragment, dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight are suitable. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg. In some methods, two or more antibodies of the invention with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Antibodies are usually administered on multiple occasions, wherein intervals between single dosages can be weekly, monthly or yearly. An exemplary treatment regime entails subcutaneous dosing, once biweekly or monthly. Advantageously, subcutaneous administration has been found to reduce the flu-like symptoms associated with intravenous infusions (Lundin, et al. (2002) Blood 100:768-773). Intervals can also be irregular as indicated by measuring blood levels of antibody to ADDLs in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μ g/mL and in some methods

- 27 -

25-300 $\mu\text{g/mL}$. Alternatively, the antibody or antibody fragment can be administered as a sustained-release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the patient. In general, human and humanized antibodies have longer half-lives than chimeric antibodies and nonhuman antibodies. As indicated above, dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, and preferably until the patient shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

Antibody and antibody fragments of the instant invention can be administered as a component of a pharmaceutical composition or medicament. Pharmaceutical compositions or medicaments generally contain the active therapeutic agent and a variety of other pharmaceutically acceptable components. See Remington: The Science and Practice of Pharmacy, Alfonso R. Gennaro, editor, 20th ed. Lippincott Williams & Wilkins: Philadelphia, PA, 2000. The preferred form depends on the intended mode of administration and therapeutic application. Pharmaceutical compositions can contain, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles commonly used to formulate pharmaceutical compositions for animal or human

- 28 -

administration. Diluents are selected so as not to affect the biological activity of the combination. Examples of such diluents are distilled water, physiological phosphate-buffered saline, Ringer's solutions, dextrose solution, and Hank's solution.

Pharmaceutical compositions can also contain large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polylactic acids, polyglycolic acids and copolymers (such as latex-functionalized SEPHAROSE™, agarose, cellulose, and the like), polymeric amino acids, amino acid copolymers, and lipid aggregates (such as oil droplets or liposomes).

Administration of a pharmaceutical composition or medicament of the invention can be carried out via a variety of routes including, but not limited to, oral, topical, pulmonary, rectal, subcutaneous, intradermal, intranasal, intracranial, intramuscular, intraocular, or intra-articular injection, and the like. The most typical route of administration is intravenous followed by subcutaneous, although other routes can be equally effective. Intramuscular injection can also be performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example, intracranial injection. In some embodiments, an antibody or antibody fragment is injected directly into the cranium. In other embodiments, antibody or antibody fragment is administered as a sustained-release composition or device, such as a MEDIPAD™ device.

For parenteral administration, antibody or antibody fragments of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a

- 29 -

pharmaceutical carrier that can be a sterile liquid such as water, oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or emulsifying agents, surfactants, pH buffering substances and the like
5 can be present in compositions. Other components of pharmaceutical compositions are those of petroleum, animal, vegetable, or synthetic origin, for example, peanut oil, soybean oil, and mineral oil. In general, glycols such as propylene glycol or polyethylene glycol are suitable liquid
10 carriers, particularly for injectable solutions. Antibodies can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained-release of the active ingredient.

15 An exemplary composition contains the instant antibody or antibody fragment formulated as a sterile, clear liquid at a concentration of at least 10 mg/ml in isotonic buffered saline (10 mM histidine, 150 mM sodium chloride, 0.01% (w/v) POLYSORBATE 80, pH 6.0). An exemplary antibody
20 formulation is filled as a single dose, 0.6 ml glass vials filled with 3.3 ml of solution per vial. Each vial is stopped with a TEFLON-coated stopper and sealed with an aluminum cap.

Typically, compositions are prepared as injectables,
25 either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid vehicles prior to injection can also be prepared. The preparation also can be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or
30 copolymer for enhanced delivery.

For suppositories, binders and carriers include, for example, polyalkylene glycols or triglycerides; such suppositories can be formed from mixtures containing the

- 30 -

active ingredient in the range of 0.5% to 10%, or more desirably 1%-2%.

Oral formulations include excipients, such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, and magnesium carbonate. These compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained-release formulations or powders and contain 10%-95% of active ingredient, or more suitably 25%-70%.

Topical application can result in transdermal or intradermal delivery. Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxins (see Glenn, et al. (1998) *Nature* 391:851). Co-administration can be achieved by using the components as a mixture or as linked molecules obtained by chemical crosslinking or expression as a fusion protein.

Alternatively, transdermal delivery can be achieved using a skin path or using transferosomes (Paul, et al. (1995) *Eur. J. Immunol.* 25:3521-24; Cevc, et al. (1998) *Biochem. Biophys. Acta* 1368:201-15).

An antibody or antibody fragment of the invention can optionally be administered in combination with other agents that are at least partly effective in treatment of amyloidogenic disease. For example, the instant antibody can be administered with existing palliative treatments for Alzheimer's Disease, such as acetylcholinesterase inhibitors such as ARICEPT™, EXELON™, and REMINYL™ and, the NMDA antagonist, NAMENDA™. In addition to these approved treatments, the instant antibody can be used to provide synergistic/additive benefit for any of several approaches currently in development for the treatment of Alzheimer's

- 31 -

Disease, which include without limitation, inhibitors of A β production and aggregation.

Antibody and antibody fragments of the instant invention also find application in the identification of therapeutic agents that prevent the binding of ADDLs to neurons (e.g., a hippocampal cell) thereby preventing downstream events attributed to ADDLs. Such an assay is carried out by contacting a neuron with ADDLs in the presence of an agent and using an antibody or antibody fragment of the invention to determine binding of the ADDLs to the neuron in the presence of the agent. As will be appreciated by the skilled artisan, an agent that blocks binding of ADDLs to a neuron will decrease the amount of ADDLs bound to the neuron as compared to a neuron which has not been contacted with the agent; an amount which is detectable in an immunoassay employing an antibody or antibody fragment of the instant invention. Suitable immunoassays for detecting neuronal-bound ADDLs are disclosed herein.

Agents which can be screened using the method provided herein encompass numerous chemical classes, although typically they are organic molecules, preferably small organic compounds having a molecular weight of more than 100 and less than about 2,500 daltons. Agents encompass functional groups necessary for structural interaction with proteins, particularly hydrogen bonding, and typically include at least an amine, carbonyl, hydroxyl or carboxyl group, preferably at least two of the functional chemical groups. The agents often contain cyclical carbon or heterocyclic structures and/or aromatic or polyaromatic structures substituted with one or more of the above functional groups. Agents can also be found among biomolecules including peptides, antibodies, saccharides,

- 32 -

fatty acids, steroids, purines, pyrimidines, derivatives, structural analogs or combinations thereof. Agents are obtained from a wide variety of sources including libraries of natural or synthetic compounds.

5 A variety of other reagents such as salts and neutral proteins can be included in the screening assays. Also, reagents that otherwise improve the efficiency of the assay, such as protease inhibitors, nuclease inhibitors, anti-microbial agents, and the like can be used. The
10 mixture of components can be added in any order that provides for the requisite binding.

 Agents identified by the screening assay of the present invention will be beneficial for the treatment of amyloidogenic diseases and/or tauopathies. In addition, it
15 is contemplated that the experimental systems used to exemplify these concepts represent research tools for the evaluation, identification and screening of novel drug targets associated with amyloid beta induction of tau phosphorylation.

20 The present invention also provides methods for detecting ADDLs and diagnosing a disease associated with accumulation of ADDLs using an antibody or antibody fragment of the instant invention. A disease associated with accumulation of ADDLs is intended to include any
25 disease wherein the accumulation of ADDLs results in physiological impairment of long-term potentiation/memory formation. Diseases of this type include, but are not limited to, Alzheimer's Disease and similar memory-related disorders.

30 In accordance with these methods, a sample from a patient is contacted with an antibody or antibody fragment of the invention and binding of the antibody or antibody fragment to the sample is indicative of the presence of

- 33 -

ADDLs in the sample. As used in the context of the present invention, a sample is intended to mean any bodily fluid or tissue which is amenable to analysis using immunoassays. Suitable samples which can be analyzed in accordance with the methods of the invention include, but are not limited to, biopsy samples and fluid samples of the brain from a patient (e.g., a mammal such as a human). For *in vitro* purposes (e.g., in assays monitoring oligomer formation), a sample can be a neuronal cell line or tissue sample. For diagnostic purposes, it is contemplated that the sample can be from an individual suspected of having a disease associated with accumulation of ADDLs or from an individual at risk of having a disease associated with accumulation of ADDLs, e.g., an individual with a family history which predisposes the individual to a disease associated with accumulation of ADDLs.

Detection of binding of the antibody or antibody fragment to ADDLs in the sample can be carried out using any standard immunoassay (e.g., as disclosed herein), or alternatively when the antibody fragment is, e.g., a peptide aptamer, binding can be directly detected by, for example, a detectable marker protein (e.g., β -galactosidase, GFP or luciferase) fused to the aptamer. Subsequently, the presence or absence of the ADDL-antibody complex is correlated with the presence or absence, respectively, of ADDLs in the sample and therefore the presence or absence, respectively, of a disease associated with accumulation of ADDLs. It is contemplated that one or more antibodies or antibody fragments of the present invention can be used in conjunction with current non-invasive immuno-based imaging techniques to greatly enhance detection and early diagnosis of a disease associated with accumulation of ADDLs.

- 34 -

To facilitate diagnosis the present invention also pertains to a kit for containing an antibody or antibody fragment of the instant invention. The kit includes a container holding one or more antibody or antibody fragments which recognizes multi-dimensional conformation of ADDLs and instructions for using the antibody for the purpose of binding to ADDLs to form an antibody-antigen complex and detecting the formation of the antibody-antigen complex such that the presence or absence of the antibody-antigen complex correlates with presence or absence of ADDLs in the sample. Examples of containers include multiwell plates which allow simultaneous detection of ADDLs in multiple samples.

The invention is described in greater detail by the following non-limiting examples.

Example 1: General Materials and Methods

ADDL Preparation. ADDLs in F12 medium (Biosource, Camarillo, CA) were prepared from A β 1-42 in accordance with established methods (Lambert, et al. (2001) *supra*). Briefly, A β 1-42 peptide (American Peptide Co., Sunnyvale, CA or California Peptide Research, Inc., Napa, CA) was weighed and placed in a glass vial capable of holding a sufficient quantity of HFIP (1,1,1,3,3,3-hexafluoro-2-propanol) to achieve a peptide concentration of 10 mg/mL. HFIP was added to the dry peptide, the vial was capped and gently swirl to mix, and the peptide/HFIP solution was stored at room temperature for at least one hour. Aliquots (50 or 100 μ L, 0.5 or 1.0 mg, respectively) of peptide solution was dispensed into a series of 1.5 mL conical centrifuge tubes. The tubes were placed in a SPEEDVAC® overnight to remove the HFIP. Tubes containing the dried

- 35 -

peptide film were capped and stored at -70°C in a sealed container with dessicant.

Prior to use, the A β 1-42 peptide film was removed from -70°C storage and allowed to warm to room temperature. Fresh DMSO (44 μ L/mg of peptide film; 5 mM) was added and the peptide/DMSO mixture was incubated on a vortex mixer at the lowest possible speed for ten minutes. F12 media (2 mL/mg peptide) was dispensed into each tube of DMSO/peptide and the tube was capped and mixed by inversion. The 100 μ M preparation was stored at 2-8°C for eighteen to twenty four hours. The samples were centrifuged at 14,000 x g for ten minutes at 2-8°C. The supernatant was transferred to a fresh tube and stored at 2-8°C until used.

Biotinylated ADDL preparations (bADDLs) were prepared in the same manner as described above for ADDL preparations using 100% N-terminal biotinylated amyloid beta peptide (American Peptide Company, Sunnyvale, CA).

Monomer Preparation. HFIP dry down preparations of amyloid beta (1-40) peptide (A β 1-40) were prepared as outlined for A β (1-42) peptide. The peptide film was dissolved in 2 mL of 25 mM borate buffer (pH 8.5) per mg of peptide, divided into aliquots, and frozen at -70°C until used.

Primary Neurons. Primary hippocampal cultures were prepared from frozen, dissociated neonatal rat hippocampal cells (Cambrex, Corp., East Rutherford, NJ) that were thawed and plated in 96-well COSTAR® plates at a concentration of 20,000 cells per well. The cells were maintained in NEUROBASAL™ media without L-glutamine (GIBCO-BRL™, Gaithersburg, MD) and supplemented with B27 (GIBCO-BRL™, Gaithersburg, MD) for a period of two weeks and then used for binding studies.

- 36 -

Immunocytochemistry. Immunocytochemistry was performed according to established methods (Lambert, et al. (2001) *supra*), except the secondary antibodies were conjugated to ALEXAFLUOR® 588 (Molecular Probes, Eugene, OR). Antibodies and ADDLs were preincubated for 1 hour at room temperature, at a molar ratio of 1:4 antibody:ADDL before application to the 21-day hippocampal cell culture. For endogenous ADDLs, human brain protein (prepared as in Lambert, et al. (2001) *supra*) was incubated with cells for 1 hour before the cells were washed, fixed, and visualized as above.

Lightly fixed frozen sections (4% paraformaldehyde at 4°C for 30 hours and cryoprotected in 40 µm sucrose) from Alzheimer's Disease and control hippocampus were incubated with antibody (1:1000 in phosphate-buffered saline (PBS)) overnight at 4°C. After removal of antibody, sections were washed 3 times with PBS and incubated with secondary antibody at room temperature. Binding was then visualized with DAB (SIGMA™, St. Louis, MO). Sections were then counterstained with hematoxylin, mounted, and imaged on a NIKON® ECLIPSE® E600 light microscope with a SPOT™ INSIGHT™ digital video camera (v. 3.2).

ELISA. Polyclonal anti-ADDLs IgG (M90/1; Bethyl Laboratories, Inc., Montgomery, TX) was plated at 0.25 mg/well on IMMULON™ 3 REMOVAWELL™ strips (Dynatech Labs, Chantilly, VA) for 2 hours at room temperature and the wells blocked with 2% BSA in TBS. Samples diluted with 1% BSA in F12 were added to the wells, allowed to bind for 2 hours at 4°C, and washed 3X with BSA/TBS at room temperature. Monoclonal antibodies diluted in BSA/TBS were incubated for 90 minutes at room temperature and detected with a VECTASTAIN® ABC kit to mouse IgG. The HRP label was visualized with BIO-RAD® peroxidase substrate and read at 405 nm on a Dynex MRX-TC microplate reader.

- 37 -

Example 2: Isolation of Mouse Antibody Variable Region Sequences

The cDNAs coding for the variable domains of the 20C2 mouse antibody were cloned and sequenced following a polymerase chain reaction (PCR) using specially designed primers that hybridize to the 5'-ends of the mouse constant regions and to the murine leader sequences upstream of the V regions. This ensured that the mouse variable region sequences obtained were complete and accurate. In short, mRNA was extracted from mouse hybridoma cell lines using the QIAGEN® OLIGOTEX® Direct mRNA Mini Kit and subsequently converted to cDNA using a first-strand cDNA synthesis kit. The cDNA was then used as template in PCR reactions to obtain the antibody variable region sequences.

To obtain the light chain variable region sequence, eleven independent PCR reactions were set up using each of the eleven light chain 5' PCR primers (MKV-1 to MKV-11) and the 3' PCR primer MKC-1 (Table 1).

TABLE 1

5' Primer	Sequence	SEQ ID NO:
MKV-1	GAT CTC TAG ATG AAG ATT GCC TGT TAG GCT GTT GGT GCT G	11
MKV-2	GAT CTC TAG ATG GAG WCA GAC ACA CTC CTG YTA TGG GTG	12
MKV-3	GAT CTC TAG ATG AGT GTG CTC ACT CAG GTC CTG GSG TTG	13
MKV-4	GAT CTC TAG ATG AGG RCC CCT GCT CAG WTT YTT GGM WTC TTG	14
MKV-5	GAT CTC TAG ATG GAT TTW CAG GTG CAG ATT WTC AGC TTC	15
MKV-6	GAT CTC TAG ATG AGG TKC YYT GYT SAY CTY CTC TGR GG	16
MKV-7	GAT CTC TAG ATG GGC WTC AAA GAT GGA GTC ACA KNY YCW GG	17
MKV-8	GAT CTC TAG ATG TGG GGA YCT KTT TYC MMT TTT TCA ATG	18
MKV-9	GAT CTC TAG ATG GTR TCC WCA SCT CAG TTC CTT G	19
MKV-10	GAT CTC TAG ATG TAT ATA TGT TTG TTG TCT ATT TCT	20
MKV-11	GAT CTC TAG ATG GAA GCC CCA GCT CAG CTT CTC TTC C	21
3' Primer	Sequence	SEQ ID NO:
MKC-1	GAT CGA GCT CAC TGG ATG GTG GGA AGA TGG	22

Underlined and italic sequences denote *Xba*I and *Sac*I restriction sites, respectively. W = A or T, M = A or C, K = G or T, Y = C or T, and R = A or G.

- 38 -

To obtain the heavy chain variable region sequences twelve independent PCR reactions were set up using each of the twelve heavy chain 5' PCR primers (MHV-1 to MHV-12) and the appropriate isotype specific 3' primer (MHCG-1, MHCG-2A, MHCG-2B, MHCG-3) (Table 2).

TABLE 2

5' Primer	Sequence	SEQ ID NO:
MHV-1	GAT <u>CTC TAG ATG AAA</u> TGC AGC TGG GGC ATS TTC TTC	23
MHV-2	GAT <u>CTC TAG ATG GGA</u> TGG AGC TRT ATC ATS YTC TT	24
MHV-3	GAT <u>CTC TAG ATG AAG</u> WTG TGG TTA AAC TGG GTT TTT	25
MHV-4	GAT <u>CTC TAG ATG RAC</u> TTT GGG YTC AGC TTG RTT T	26
MHV-5	GAT <u>CTC TAG ATG GGA</u> CTC CAG GCT TCA ATT TAG TTT TCC TT	27
MHV-6	GAT <u>CTC TAG ATG GCT</u> TGT CYT TRG SGC TRC TCT TCT GC	28
MHV-7	GAT <u>CTC TAG ATG GRA</u> TGG AGC KGG RGT CTT TMT CTT	29
MHV-8	GAT <u>CTC TAG ATG AGA</u> GTG CTG ATT CTT TTG TG	30
MHV-9	GAT <u>CTC TAG ATG GMT</u> TGG GTG TGG AMC TTG CTT ATT CCT G	31
MHV-10	GAT <u>CTC TAG ATG GGC</u> AGA CTT ACC ATT CTC ATT CCT G	32
MHV-11	GAT <u>CTC TAG ATG GAT</u> TTT GGG CTG ATT TTT TTT ATT G	33
MHV-12	GAT <u>CTC TAG ATG ATG</u> GTG TTA AGT CTT CTG TAC CTG	34
3' Primer	Sequence	SEQ ID NO:
MHCG-1	GCATC GAG CTC CAG TGG ATA GAC AGA TGG GGG	35
MHCG-2A	GCATC GAG CTC CAG TGG ATA GAC CGA TGG GGG	36
MHCG-2B	GCATC GAG CTC CAG TGG ATG AGC TGA TGG GGG	37
MHCG-3	GCATC GAG CTC CAA GGG ATA GAC AGA TGG GGC	38

Underlined and italic sequences denote *Xba*I and *Sac*I restriction sites, respectively. W = A or T, M = A or C, K = G or T, Y = C or T, and R = A or G.

10

Each of the light chain PCR reactions contained 46 μ L INVITROGEN™ PLATINUM® PCR Super Mix, 1.0 μ L of one of the 100 μ M 5' primers (MKV-1 to MKV-11), 1.0 μ L of the 100 μ M 3' primer (MKC-1), and 2.0 μ L of hybridoma cDNA. Similar PCR reactions were employed to clone the mouse heavy chain variable region sequences. Reactions were placed in a DNA thermal cycler and, after an initial denaturation step at 97°C for 2.0 minutes, subjected to 30 cycles of: 95°C for 30 seconds, 55°C for 45 seconds, and 72°C for 90 seconds. Following the last cycle, a final extension step at 72°C for 10 minutes was employed. To determine which PCR reactions yielded product, 5 μ L aliquots from each reaction

15

20

- 39 -

were separated on 1.5% (w/v) agarose/1X TAE buffer gels, containing 0.5 µg/mL ethidium bromide. PCR products from reactions that produced fragments of the expected size (420 to 500 bp) were then gel purified, digested with *Xba*I and *Sac*I and ligated into the *Xba*I and *Sac*I sites in the multicloning region of plasmid pNEB193 (New England Biolabs, Beverly, MA). Alternatively, PCR products were ligated directly into plasmid pCR®2.1 using the INVITROGEN™ TA CLONING® kit. Ligation products were then transformed into XL-1 cells and aliquots of the transformed *E. coli* were plated onto LB agar plates containing 50 µg/mL ampicillin and overlaid with 40 µL of X-Gal stock (50 mg/mL) and 40 µL IPTG (100 mM) solution for blue/white selection. Plates were incubated overnight at 37°C and potential clones were identified as white colonies. DNA from at least 24 independent clones for each PCR product were sequenced on both strands using universal forward and reverse primers for pNEB193 and pCR®2.1. The resulting sequences were then assembled into a contig to generate a consensus sequence for each antibody light and heavy chain variable region. Using this approach the sequences were determined for the light and heavy antibody variable regions of hybridoma 20C2 (Figures 1A-1B). The six complementarity-determining regions (CDRs), which form the structure complementary to the antigen, are underlined in Figures 1A-1B.

Example 3: Humanization of Mouse Anti-ADDL Antibody Variable Region Sequences

Mouse antibody heavy and light variable domain nucleic acids obtained from mouse hybridoma cell line 20C2 were humanized using a CDR grafting approach. It will be appreciated by those skilled in the art that humanization

- 40 -

of mouse antibody sequences can maximize the therapeutic potential of an antibody by improving its serum half-life and Fc effector functions--thereby reducing the anti-globulin response.

5 Humanization by CDR grafting was carried out by selecting the human light and heavy chain variable regions from the NCBI protein database with the highest homology to the mouse variable domains. The mouse variable region sequences were compared to all human variable region
10 sequences in the database using the protein-protein Basic Local Alignment Search Tool (BLAST). Subsequently, mouse CDRs were joined to the human framework regions and the preliminary amino acid sequence was analyzed. All differences between the mouse and human sequences in the
15 framework regions were evaluated particularly if they were part of the canonical sequences for loop structure or were residues located at the VL/VH interface (O'Brien and Jones (2001) *In: Antibody Engineering*, Kontermann and Dubel (Eds.), Springer Laboratory Manuals). Framework regions
20 were also scanned for unusual or rare amino acids in comparison to the consensus sequences for the human subgroup and for potential glycosylation sites. Wherein amino acid sequence differences existed between the mouse and human framework region sequences that were not found to
25 be involved in canonical sequences, or located at the VL/VH interface, the human residue was selected at that position. Wherein a difference in a key residue existed, two versions of the variable region sequence were generated for evaluation. The CDR grafting strategy made the minimum
30 number of changes to the human framework region so that good antigen binding was achieved while maintaining human framework regions that closely matched the sequence from a natural human antibody. The heavy chain and light chain

- 41 -

variable region amino acid sequences of the resulting humanized antibody generated by CDR grafting of murine 20C2 are shown in Figures 2A and 2B, respectively. This antibody is designated herein as Hu20C2.

5 Humanized sequences for 20C2 were also designed using a veneering strategy (See, e.g., U.S. Patent No. 6,797,492). Humanization was carried out by selecting the human light and heavy chain variable regions from the NCBI protein database with the highest homology to the mouse
10 variable domains, as well as to the closest human antibody germline family or families (see, Kabat, et al. (1991) Sequences of proteins of immunological interest, 5th ed., U.S. Dept. Health and Human Services, NIH, Washington DC). The mouse variable region sequences were compared to all
15 human variable region sequences in the database using protein-protein BLAST. The murine variable sequences and their closest human homologues were modeled to the closest crystallized human antibody as determined by computer modeling as practiced in the art. From the model of the
20 murine VH and VL sequences, a surface area map was constructed, which dictated the solvent accessibility of the amino acids in the mouse heavy and light variable regions. To confirm the modeling, these exposed residues were compared position-by-position with known surface
25 accessible residues (see, e.g., Padlan (1994) *Mol. Immunol.* 31(3):169-217). A score was assigned for each residue in the sequence designating it as exposed, mostly exposed, partly buried, mostly buried and buried according to established methods (see, U.S. Patent No. 6,797,492, incorporated
30 herein by reference in its entirety). Mouse framework residues that scored as exposed or mostly exposed and differed from the homologous human sequence were changed to the human residue at that position. The designed veneered

- 42 -

sequences retained the mouse CDRs, residues neighboring the CDRs, residues known to be involved in canonical sequences, residues located at the VL/VH interface, and residues at the N-terminal sequences of the mouse heavy and light chain. The N-terminal sequences are known to be contiguous with the CDR surface and are potentially involved in ligand binding. Once the veneered sequences were finalized they were remodeled to look for any potential obvious structural issues. A total of 12 and 9 amino acid residues were changed in the heavy chain and light chain frames, respectively. The heavy chain and light chain variable region amino acid sequences of the resulting humanized antibody generated by veneering of murine 20C2 are shown in Figures 2A and 2B, respectively. This antibody is designated herein as Hu20C2A3.

In comparison to 20C2, it is noted that the light chain substitutions resulting in Hu20C2A3, but not the heavy chain substitutions, are in common with Hu20C2 (Figure 2). In particular, heavy chain variable region CDR3 is unique to Hu20C2A3.

Once the humanized amino acid sequences were selected the sequences were reverse-translated to obtain the corresponding DNA sequence. The DNA sequences were codon-optimized using art-established methods (Lathe (1985) *J. Mol. Biol.* 183(1):1-12) and designed with flanking restriction enzyme sites for cloning into human antibody expression vectors. The nucleotide sequences encoding the light chain variable region and two versions of the heavy chain variable region for Hu20C2 are presented in Figures 3A-3C. The two heavy chain variable region versions differ by a single amino acid substitution at position 24; heavy chain variable region for version A of Hu20C2 is Phe at

- 43 -

position 24 and heavy chain variable region of version B of Hu20C2 is Leu at position 24.

Example 4: Affinity Maturation

5 Affinity maturation was carried out on the Hu20C2 antibody. Nucleic acid molecules encoding humanized Hu20C2 versions A and B variable heavy chain only, light chain only or heavy chain version A and light chain together were cloned in the Fab phage-display vector pFab4. Nucleic acid
10 sequence analysis confirmed sequence and orientation in pFab4. The annotated Hu20C2 Fab sequences in pFab4 are presented in Figures 4A-4C and set forth herein as SEQ ID NO:116 for heavy chain version A, SEQ ID NO:117 for heavy chain version B, and SEQ ID NO:118 for the light chain. The
15 nucleotide sequence for heavy chain version A and light chain together in the pFab4 vector is presented in Figures 4D-4E. These constructs were used in the Hu20C2 maturation program using art-established phage-displayed Fab library methods.

20 *Light Chain Maturation.* Two libraries were designed to mutate the nine wild-type amino acids of CDR3 of the light (kappa) chain of Hu20C2 (i.e., Phe-Gln-Gly-Ser-Leu-Val-Pro-Leu-Thr; SEQ ID NO:39). These libraries were designated LC3-1 and LC3-2 representing light chain CDR3 sequences of
25 Xaa-Xaa-Xaa-Xaa-Xaa-Val-Pro-Leu-Thr (SEQ ID NO:40) and Phe-Gln-Gly-Ser-Xaa-Xaa-Xaa-Xaa-Xaa (SEQ ID NO:41), respectively. Biotinylated reverse primers, 20C2LC3-1 (SEQ ID NO:123) and 20C2LC3-2 (SEQ ID NO:126), were used in combination with forward primer 20C2LC3F (SEQ ID NO:120) to
30 generate the LC3-1 and LC3-2 libraries (see Figure 5A). Primers were purified by polyacrylamide gel electrophoresis, whereas the vector DNA was purified by gel electrophoresis and electroelution. The two light chain

- 44 -

libraries were designed to be randomly mutated. The final diversities of the three 10G5H6 LC₃ libraries were 4.76×10^8 and 7.45×10^8 , respectively (Table 3). Sequence analysis of approximately 100 clones from the libraries showed 100% diversity of mutant clones at the designed amino acid positions.

TABLE 3

Characteristic	Light Chain Library	
	LC3-1	LC3-2
Vector	pFab3d20C2HS	pFab3d20C2HS
Number of Transformants	4.76×10^8	7.45×10^8
Library Diversity	$4.76 \times 10^8 \times 0.89 = 4.24 \times 10^8$	$7.45 \times 10^8 \times 0.90 = 6.71 \times 10^8$
Primary Library Volume	2 mL	2 mL
Primary Library Titer	2.13×10^{11}	$*9.3 \times 10^{10}$

*Higher titers are achieved by concentration or phage rescue.

10

Soluble panning of the two light chain libraries against high molecular weight bADDL was completed. Briefly, four rounds of panning were carried out using biotinylated high molecular weight ADDL (bADDL). The first three rounds were carried out using approximately 1.5 μ M antigen concentration (input = 1×10^{10} to 1×10^{11}). Upon completion of the third round, the outputs of the two libraries were combined and divided into three groups for analysis with 10 nM, 100 nM and approximately 1.5 μ M antigen to increase panning stringency. As such, a total of 58 output plates were tested in phage ELISA assays, i.e., two plates per library in the first round (a total of four plates), six plates per library in the second round (a total of 12 plates), eight plates for LC3-1 and 10 plates for LC3-2 libraries in the third round (a total of 18 plates) and

25

- 45 -

eight plates for each antigen concentration in the fourth round (a total of 24 plates).

Panning resulted in 1000 hits, 436 of which were sequenced (Table 4).

5

TABLE 4

Round	Antigen	Input	Output	% Recovery	ELISA Screen*	Sequenced
1 ^a	1.6 μ M	2.13×10^{10}	7.3×10^4	3.42×10^{-6}	0% (0/176)	0
2 ^a	2.0 μ M	1.55×10^{11}	1.88×10^5	1.21×10^{-6}	1.5% (8/528)	8
3 ^a	1.1 μ M	1.80×10^{10}	7.8×10^4	4.3×10^{-6}	5.8% (41/704)	41
1 ^b	1.6 μ M	9.30×10^9	5.7×10^4	6.13×10^{-6}	2.3% (7/176)	4
2 ^b	2.0 μ M	1.23×10^{11}	1.07×10^5	8.7×10^{-7}	4.5% (24/528)	24
3 ^b	1.1 μ M	1.37×10^{10}	3.32×10^5	2.42×10^{-5}	15% (134/880)	134
4 ^c	1.1 μ M	3.0×10^{11}	1.37×10^5	4.6×10^{-7}	39% (274/704)	-
4 ^c	100 nM	3.0×10^{11}	3.88×10^5	1.29×10^{-6}	41% (290/704)	-
4 ^c	10 nM	3.0×10^{11}	1.6×10^5	5.3×10^{-7}	32% (225/704)	225
Total					1000/5104	436

^a20C2 LC3-1 versus high molecular weight 10% bADDL.

^b20C2 LC3-2 versus high molecular weight 10% bADDL.

^c20C2 LC3-1 + 20C2 LC3-2 versus high molecular weight 10% bADDL.

10 *Hits per total number of colonies.

Sequence and frequency of highly enriched clones are presented in Table 5.

TABLE 5

Clone Designation	LC CDR3	SEQ ID NO:	Round 2	Round 3	Round 4	Total
Hu20C2LC	FQGS LVPLT	39	6	15	14	35
SJ-p1-31	ADTTHVPLT	42		1	2	3
SJ-p1-14	AHSTFVPLT	43	1	1	2	4
4P2-12-E3	AQASFVPLT	44			2	2
SJ-p1-38	AQATKVPLT	45		1	1	2
4P3-59	AQSSKVPLT	46			2	2
SJ-p2-14	AQSTLVPLT	47		1	2	3
4P3-11	FAASSVPLT	48			2	2
4P3-1	FESTYVPLT	49			2	2
SJ-p2-10	FESSRVPLT	50		1	1	2
SJ-p2-11	FNATWVPLT	51		2		2

- 46 -

SJ-p2-60	FQASRVPLT	52		1	5	6
SJ-p1-18	FQATRVPLT	53		1	5	6
SJ-p3-51	FQGSFIGLS	54	1		1	2
SJ-p3-16	FQGSFIPGT	55		2	3	5
SJ-p8-8F	FQGSFLPPS	56		1	1	2
SJ-p3-26	FQGSFLPQL	57	1	2		3
SJ-p3-15	FQGSFLFPV	58	1	2		3
SJ-p2-70	FQGSFLFSPS	59	1	5		6
SJ-p3-24	FQGSRIPIIS	60		1	1	2
SJ-p3-33	FQGSRLPVS	61		2	3	5
SJ-p3-14	FQGSRVPLV	62		2	1	3
SJ-p2-1F	FQSSFVPLT	63		6	8	14
4P1-22	FQSSRVPLT	64			15	15
SJ-p2-44	GQTTLVPLT	65		1	3	4
SJ-p1-56	HESTLVPLT	66		2	1	3
4P1-40	HQSSKVPLT	67			4	4
SJ-p2-20	IQTSLVPLT	68		2		2
SJ-p1-41	IQAALVPLT	69		1	1	2
SJ-p2-13	LQSSFVPLT	70	1	4		5
4P1-26	LETSRVPLT	71			3	3
SJ-p1-33	LASSHVPLT	72		2	1	3
SJ-p2-27	LNSTTVPLT	73		2	4	6
SJ-p2-62	LQSKSVPLT	74		2		2
4P2-26-E5	LQSVRVPLT	75			3	3
4P1-32	LQSSLVPLT	76			5	5
SJ-p2-37	LQTGRVPLT	77		2	2	4
SJ-p2-64	LQTSFVPLT	78		3		3
4P1-20	LQTSNVPLT	79			5	5
SJ-p2-39	LQTRVPLT	80		2	6	8
SJ-p2-52	LSSTFVPLT	81		3	1	4
SJ-p2-6L	LSSTHVPLT	82		2	1	3
4P1-77	LTSSAVPLT	83			2	2
SJ-p1-59	LVSSLVPLT	84		2		2
SJ-p2-23	METANVPLT	85		2		2
SJ-p1-9M	MQSSFVPLT	86		1	3	4
SJ-p2-28	MQSSLVPLT	87		1	2	3
SJ-p1-21	MQTSKVPLT	88		1	1	2
4P1-17	SQARMVPLT	89			3	3
SJ-p2-66	SQASRVPLT	90		1	2	3
SJ-p1-49	TQSTQVPLT	91		2	1	3
SJ-p2-24	VCATFVPLT	92		1	1	2
4P1-41	VQSSAVPLT	93			2	2
SJ-p2-51	VQTSLVPLT	94		12	31	43
4P1-64	VQTSVVPLT	95			3	3
SJ-p2-55	VQTTAVPLT	96		2		2
SJ-p1-25	LQTARVPLT	97		1	3	4

Fab fragments from the 10 top clones based on enrichment frequency were prepared and a total of 15 clones were converted into IgG1 humanized A version and two clones, 20C2-6 and 20C2-8, were converted to IgG1 humanized

- 47 -

B version. K_D values for these clones were measured by BIACORE™ using biotin-A β 1-20 (Table 6) and bADDL (Table 7) as antigens. Dramatic improvements in affinity were observed as compared to parental humanized 20C2A and 20C2B, as well as mouse 20C2 antibodies. In particular, low nanomolar to sub-picomolar K_D s were achieved with a light chain CDR3 of the sequence Xaa₁-Gln-Xaa₂-Thr-Arg-Val-Pro-Leu-Thr (SEQ ID NO:2), wherein Xaa₁ is Phe or Leu, and Xaa₂ is Ala or Thr. Moreover, a comparison between K_D values obtained with BIACORE™ using biotin-A β 1-20 and bADDL further demonstrates that anti-ADDL antibodies such as Hu20C2 preferentially bind multi-dimensional conformations of ADDLs over monomeric A β peptides.

15

TABLE 6

Name	Clone	LC-CDR3	SEQ ID NO:	K_D (Biotin-A β 1-20)		
				Fab	IgG1#1	IgG1#2
20C2-1A	SJ-p2-60	FQASRVPLT	52	91 nM	1.2 nM	--
20C2-2A	SJ-p1-18	FQATRVPLT	53	28 nM	686 pM	2 nM
20C2-3A	SJ-p3-16	FQGSFIPGT	55	--	1.7 nM	--
20C2-5A	SJ-p2-1F	FQSSFVPLT	63	41 nM	912 pM	1.5 nM
20C2-6A	4P1-22	FQSSRVPLT	64	18 nM	544 pM	714 pM
20C2-6B	4P1-22	FQSSRVPLT	64	--	53 pM	--
20C2-7A	SJ-p2-27	LNSTTVPLT	73	128 nM	--	--
20C2-8A	SJ-p2-39	LQTTRVPLT	80	14 nM	140 pM	376 pM
20C2-8B	SJ-p2-39	LQTTRVPLT	80	--	46 pM	64 pM
20C2-9A	SJ-p2-51	VQTSLVPLT	94	36 nM	241 pM	420 pM
20C2-10A	SJ-p3-33	FQGSRLPVS	61	--	84 nM	--
20C2-11A	SJ-p3-6	FQGSLLPLS	98	--	--	--
20C2-12A	4P1-32	LQSSLVPLT	76	617 nM	1.5 nM	--
20C2-13A	4p1-20	LQTSNVPLT	79	94 nM	3 nM	--
20C2-18A	SJ-p1-9M	MQSSFVPLT	86	126 nM	1.8 nM	--
20C2-20A	SJ-p3-15	FQGSFPPV	58		21 nM	
20C2-22A	SJ-p2-66	SQASRVPLT	90		2.3 nM	
20C2-23A	4P1-40	HQSSKVPLT	67		649 pM	1.5 nM
20C2-24A	SJ-p2-44	GQTTLVPLT	65		1.9 nM	
20C2A		FQGSFVPLT	39		27 nM	
20C2B		FQGSFVPLT	39		5.4 nM	
Mouse-20C2		FQGSFVPLT	39	83 nM	3.4 nM	

- 48 -

TABLE 7

Name	Clone	LC-CDR3	SEQ ID NO:	K _D (bADDL)		
				Fab	IgG1#1	IgG1#2
20C2-1A	SJ-p2-60	FQASRVPLT	52	85 nM	75 pM	--
20C2-2A	SJ-p1-18	FQATRVPLT	53	28 nM	15 pM	0.3 pM
20C2-3A	SJ-p3-16	FQGSFIPGT	55	--	3.7 nM	--
20C2-5A	SJ-p2-1F	FQSSFVPLT	63	41 nM	317 pM	68 pM
20C2-6A	4P1-22	FQSSRVPLT	64	42 nM	4.3 pM	24 pM
20C2-6B	4P1-22	FQSSRVPLT	64	--	53 pM	--
20C2-7A	SJ-p2-27	LNSTTVPLT	73	435 nM	--	--
20C2-8A	SJ-p2-39	LQTTRVPLT	80	13 nM	3 pM	0.7 pM
20C2-8B	SJ-p2-39	LQTTRVPLT	80	--	13 pM	0.8 pM
20C2-9A	SJ-p2-51	VQTSLVPLT	94	40 nM	--	2 pM
20C2-10A	SJ-p3-33	FQGSRLPVS	61	--	7.7 nM	
20C2-11A	SJ-p3-6	FQGSLLPLS	98	--	--	--
20C2-12A	4P1-32	LQSSLVPLT	76	238 nM	15 pM	--
20C2-13A	4p1-20	LQTSNVPLT	79	567 nM	764 pM	
20C2-18A	SJ-p1-9M	MQSSFVPLT	86	85 nM	149 pM	
20C2-20A	SJ-p3-15	FQGSLEFPV	58		6.9 nM	
20C2-22A	SJ-p2-66	SQASRVPLT	90		198 pM	
20C2-23A	4P1-40	HQSSKVPLT	67		85 pM	66 pM
20C2-24A	SJ-p2-44	GQTTLVPLT	65		114 pM	
20C2A		FQGSIVPLT	39			
20C2B		FQGSIVPLT	39			
Mouse-20C2		FQGSIVPLT	39	62 nM	4.1 nM	

Heavy Chain Maturation. The heavy chain of Hu20C2 was also subjected to optimization by generation of 3 libraries covering the heavy chain-CDR3 (RQLGLRSIDAMDY; SEQ ID NO:99). These libraries were designated 20C2B-39HC₃-1, 20C2B-39HC₃-2, and 20C2B-39HC₃-3 representing heavy chain CDR3 sequences of XXXXXRSIDAMDY (SEQ ID NO:100) and RQLGLRSIXXXXX (SEQ ID NO:101) and RQLGXXXXXAMDY (SEQ ID NO:102), respectively. Biotinylated reverse primers, 20C2HC3-1 (SEQ ID NO:130), 20C2HC3-2 (SEQ ID NO:133), and 20C2HC3-3 (SEQ ID NO:136) were used in combination with forward primer 20C2HC3F (SEQ ID NO:127) to generate the 3 libraries (see Figure 5B). The libraries contained >10⁸ functional diversity and covered all combinations of amino acids at every position randomized in each set (see Table 8).

- 49 -

TABLE 8

Monoclonal Antibody	Diversity	Sequence	SEQ ID NO:
Hu20C2		RQLGLRSIDAMDY	99
20C2B-39HC ₃ -1	5.78 x 10 ⁸	XXXXXRSIDAMDY	100
20C2B-39HC ₃ -2	6.16 x 10 ⁸	RQLGLRSIXXXXX	101
20C2B-39HC ₃ -3	3.99 x 10 ⁸	RQLGXXXXXAMDY	102

A total of 18 output plates from 4 rounds of panning were tested in a phage ELISA assay. A total of 1235 hits were found, of which 704 were sequenced. Based on BIACORE™ K_D values of Fab fragments against biotinylated-Aβ1-20 and biotinylated ADDL (bADDL) antigens, as well as single-point BIACORE™3000 analysis (Table 9), a total of 6 Fab clones were converted into IgG1 and IgG2m4 using either CDR grafting or veneering humanization techniques. One of these 6 Fabs, designated 4a-A3, was isolated from library 20C2B-39HC₃-3 and carried 3 amino acid substitutions (RQLGTRGTDAMDY; SEQ ID NO:3) in the middle section of the heavy chain. As is evident from Figure 2B, this heavy chain CDR3 sequence is that of Hu20C2A3.

TABLE 9

Clone	bADDL Binding K _D (M)	Aβ 1-20 Binding K _D (M)	BIACORE™ 3000 Off-rate	Sequence	SEQ ID NO:
4a-A3	8.23E-11	8.49E-10	6.65E-05	RQLGTRGTDAMDY	3
4b-A7	3.76E-10	1.72E-09	1.23E-04	RQLGKLALDAMDY	142
4b-H11	1.05E-09	1.33E-09	1.32E-04	RQLGRRSVDAMDY	8
20C28B	1.10E-09	1.10E-09	8.76E-05	RQLGLRSIDAMDY	143
4a-F5	1.39E-09	1.33E-09	1.17E-04	RQLGKLKTDAMDY	7
4a-B2	1.92E-09	1.29E-09	1.13E-04	RQLGARKTDAMDY	5
4b-D8	2.23E-09	1.69E-09	1.45E-04	RALSPRSIDAMDY	4
4a-A4	2.67E-09	1.58E-09	1.20E-04	RALSPRSIDAMDY	4
4b-A1	2.87E-09	2.85E-09	1.23E-04	RQLGPRKRDAMDY	6
4a-A7	3.24E-09	2.21E-09	1.55E-04	RQLGQRQTDAMDY	144
4a-B9	3.44E-09	3.54E-09	1.94E-04	RAIQPRSIDAMDY	145
4a-B3	4.17E-09	3.64E-09	1.59E-04	RQLGLRSIDAHTR	146
4a-G10	4.52E-09	2.72E-09	1.78E-04	RQLGQPSVDAMDY	147
4a-E11	4.93E-09	3.48E-09	1.65E-04	RQLGFQSTDAMDY	148

- 50 -

4a-C9	8.43E-09	2.46E-09	1.75E-04	RQLGQAGHDAMDY	149
4a-D5	1.17E-09	3.91E-09	1.74E-04	RQLGDNVADAMDY	150
4a-E10	1.85E-08	3.60E-09	1.39E-04	RQLGFQSTDAMDY	148
4b-D4	1.86E-08	4.87E-09	1.89E-04	RQLGMATPDAMDY	151
4b-B10	6.28E-08	7.43E-09	1.81E-04	RQLGAHWLDAMDY	152
4b-A12	1.54E-07	1.01E-08	1.69E-04	RQLGPEPQDAMDY	153

Example 5: Generation of IgG2m4 Antibodies

IgG2m4 antibody derivatives were prepared to decrease Fc receptor engagement, C1q binding, unwanted cytotoxicity or immunocomplex formation while maintaining both the long half-life and pharmacokinetic properties of a typical human antibody. The basic antibody format of IgG2m4 is that of IgG2, which has been shown to possess a superior half-life in experimental models (Zuckier, et al. (1994) *Cancer Suppl.* 73:794-799). The structure of IgG2 was modified to eliminate C1q binding, through selective incorporation of IgG4 sequences, while maintaining the typical low level of FcγR binding (Canfield and Morrison (1991) *J. Exp. Med.* 173:1483-1491). This was achieved by using cross-over points wherein sequences of IgG2 and IgG4 were identical, thereby producing an antibody containing natural Fc sequences rather than any artificial mutational sequences. The advantages of using the instant IgG2m4 antibody which exhibits minimal effector-related activity is comparable to the deglycosylated antibody disclosed by Wilcock et al. ((2006) *J. Neurosci.* 26:5340-6).

The IgG2m4 form of the human antibody constant region was formed by selective incorporation of human IgG4 sequences into a standard human IgG2 constant region, as shown in Figure 6. Conceptually, IgG2m4 resulted from a pair of chain-swaps within the CH2 domain as shown in Figure 6. Four single mutations were made corresponding to sequences from IgG4. The Fc residues mutated in IgG2 included His268Gln, Val309Leu, Ala330Ser, and Pro331Ser,

- 51 -

which minimized the potential for neoepitopes. The specific IgG4 amino acid residues placed into the IgG2 constant region are shown in Table 10, along with other alternatives from the basic structure.

5

TABLE 10

Residue (Kabat numbering)	Residue in IgG2	Residue in IgG4	Residue in IgG2m4	Alternative residue in IgG2m4	Comment
189	Pro or Thr*	Pro	Thr	Pro	Key polymorphism of IgG2; Pro residue present in IGHG*01 allotype and Thr residue present in IGHG2*02 allotype ^{a,b} .
268	His	Gln	Gln	--	Change in the B/C loop known to be involved in FcγRII binding ^c .
309	Val	Leu or Val	Leu	Val	FcRn binding domain
330	Ala	Ser	Ser	--	Key residue for C1q binding ^d ; also potentially involved in binding FcγRII and FcγRIII ^e .
331	Pro	Ser	Ser	--	Key residue for C1q binding ^{d,f} and FcγRI binding ^g ; also potentially involved in binding FcγRII and FcγRIII ^e .
397	Met or Val*	Val	Met	Val	Val residue present in IGHG*01 allotype and Met residue present in IGHG2*02 allotype ^a .

*Positions marked with an asterisk are subject to allelic variations.

^aHougs, et al. (2001) *Immunogenetics* 52(3-4):242-8.

^bWO 97/11971.

10 ^cMedgyesi, et al. (2004) *Eur. J. Immunol.* 34:1127-1135.

^dTao, et al. (1991) *J. Exp. Med.* 173:1025-1028.

^eArmour, et al. (1999) *Eur. J. Immunol.* 29:2613.

^fXu, et al. (1994) *J. Biol. Chem.* 269:3469-3474.

^gCanfield and Morrison (1991) *J. Exp. Med.* 173:1483.

15

Human IgG1/kappa and IgG2m4/kappa versions of humanized Hu20C2 and Hu20C2A3 antibodies were constructed. The complete amino acid sequence of the light and heavy

- 52 -

chain Hu20C2A3 IgG2m4 antibody is shown in Figures 7A and 7C.

**Example 6: Binding Affinity and Specificity of Humanized
5 Anti-ADDL Antibodies**

Affinity maturation was carried out to improve affinity and improve preferential binding to ADDL. To evaluate ADDL binding affinity of the humanized antibodies, BIAcore™ and titration ELISAs were conducted as disclosed
10 herein. Briefly, Streptavidin-coated, 96-well microtiter plates (Sigma, St. Louis, MO) were coated with 10% biotinylated ADDL antigen (1 μM). A series of 2-fold dilutions of purified antibody, starting at 500 ng/mL was added to the ADDL captured plates and the plates were
15 incubated for 2 hours at 25°C. After washing five times with PBS solution using a plate washer (Bio-Tek, Winooski, VA), polyclonal goat anti-human kappa light chain antibody (Biomedex, Foster City, CA) was added at a 1/2000 dilution in 3% non-fat milk blocker and incubated at room
20 temperature for 1 hour. A rabbit anti-goat IgG (H+L) HRP-conjugated (Bethyl Laboratories, Inc., Montgomery, TX) detection antibody was then added at a 1/2000 dilution in blocking solution and incubated for 1 hour at room temperature. After washing with PBS, HRP substrate,
25 3,3',5,5'-tetramethylbenzidine (ready-to-use TMB; Sigma, St. Louis, MO) was added and the reaction was stopped after 10 minutes with 0.5 N H₂SO₄. Absorbance at wavelength of 450 nm was read in a plate reader (model VICTOR V; Perkin Elmer, Boston, MA) and data were processed using EXCEL® work
30 sheet. Assay variations between plates were estimated within 20%.

The K_D of Fab clone A3, as measured by BIAcore™, was 849 pM against biotinylated-Aβ1-20. The K_D of the same Fab

- 53 -

clone was 82 pM against bADDLs, indicating that the A3 Fab demonstrated preferential binding to ADDLs. When the clone was humanized by veneering and converted to a full IgG1 molecule or full IgG2m4 molecule (*i.e.*, Hu20C2A3), the K_D values against A β 1-20 and ADDL were below the reliable detection limit of the BIAcore™ instrument, indicating a significant improvement of Hu20C2A3's binding equilibrium constant against A β 1-20 and ADDL as compared to Hu20C2.

Hu20C2A3, which is the veneered version of clone A3 with an IgG2m4 isotype, was expressed in both CHO and *Pichia*. The two sources of Hu20C2A3 were evaluated for their ability to interact with A β monomer and ADDLs by ELISA. As shown in Figure 8, Hu20C2A3 produced in either CHO (Figure 8A) or *Pichia* (Figure 8B) showed preferential ADDL binding versus A β 40 monomer binding (6-fold). Binding constants (IgG K_{50} values) as determined from these curves yielded values of 64 pM and 376 pM for ADDLs and A β monomer, respectively for Hu20C2A3 produced in *Pichia* and 58 pM and 361 pM for ADDLs and A β monomer, respectively for Hu20C2A3 produced in CHO cells.

Example 7: Inhibition of ADDL Binding to Neurons Using Humanized Anti-ADDL Antibodies

The humanized anti-ADDL antibodies were further evaluated for their ability to block ADDL binding to primary hippocampal neurons using the methods disclosed herein. Hu20C2A3 antibody, or PBS as a control, was mixed at various molar ratios with bADDLs and incubated for one hour at 37°C on a slow rotator. After the preincubation, the antibody/bADDL preparations were added to primary neuron cultures and incubated for an additional hour at 37°C. At the end of the incubation period, the bADDLs/antibody mixture was removed and the plates washed

- 54 -

six times with media. The cells were then fixed in 4% paraformaldehyde for ten minutes at room temperature, the solution removed, fresh fixative added, and the cells fixed for an additional ten minutes. The cells were permeabilized with 4% paraformaldehyde containing 0.1% TRITON™ X-100 (2 times, each for ten minutes at room temperature), washed six times in PBS and then treated with 10% BSA in PBS for one hour at 37°C. Alkaline phosphatase-conjugated streptavidin (1:1,500 in 1% BSA; Molecular Probes, Eugene, OR) was then added to the cells for one hour at room temperature. The cells were rinsed six times with PBS, the alkaline phosphatase substrate (CDP-STAR® with SAPPHIRE-II™; Applied Biosystems, Foster City, CA) added to the cells and incubated for thirty minutes prior to determining the luminescence on a LJJL Luminometer (Analyst AD; LJJL Biosystems, Sunnyvale, CA). In this analysis, Hu20C2A3 was found to effectively inhibit bADDL binding to neurons at a sub-stoichiometric antibody to peptide ratio ($EC_{50} = 0.16$; Figure 9).

The inhibition of bADDL binding indicated that Hu20C2A3 interacts with ADDLs in a biologically relevant manner. To demonstrate that Hu20C2A3 interacts with ADDLs relevant to the human Alzheimer's Disease condition, the ability of biotinylated Hu20C2A3 to immuno-label A β containing plaques in human Alzheimer's Disease brain tissue was evaluated. Immunohistochemical localization showed avid labeling of A β in both dense core and diffuse plaques in human Alzheimer's Disease brain tissue. The specificity of this binding was demonstrated by a loss of immunoreactivity following pre-incubation with increasing ADDL:antibody amounts. Similar to Hu20C2, Hu20C2A3, efficiently labels both dense core and diffuse A β deposits.

- 55 -

Example 8: Thermal Stability of Hu20C2A3

An evaluation of the protein stability of Hu20C2A3 was assessed using SEC-HPLC, fluorescent thermal melt analysis, and particle size analysis. Fluorescent thermal melt analysis indicated that Fc and Fab unfolding transitions occurred at approximately 70°C and 80°C, respectively, consistent with acceptable inherent protein stability (Figure 10).

10 Example 9: In Vivo Pharmacodynamic and Efficacy Analysis

The prior art indicates that systemic injection of monoclonal anti-A β antibodies can increase plasma levels of A β acutely, whereas measurable lowering of brain A β requires chronic administration. It has been suggested that passive immunization in species with measurable A β results in an elevation of plasma A β due to a change in the equilibrium of A β between brain and peripheral compartments. This "peripheral sink" ultimately leads to lowering of brain A β . However, cognitive improvement has been observed in animals following acute antibody administration prior to notable changes in brain A β , indicating that changes in brain A β may occur in some form prior to a time point where these changes can be measured using known techniques. Alternately, elevations of plasma A β could be explained by a stabilization of peripheral A β following administration of antibody. Regardless of the interpretation, it has been established that early plasma elevations are a prerequisite for subsequent lowering of brain A β in animal models. Thus, the effect of Hu20C2A3 antibodies on plasma A β elevations were utilized as an indicator of target engagement. Following infusion of Hu20C2A3 at doses of 30, 100 and 300 μ g/mouse IV, significant and robust increases in plasma A β x-40 were

- 56 -

observed relative to the non-relevant antibody (8B4) control group 4 hours post-injection (Figure 11). The observed increases in plasma A β x-40 for CHO-derived material were 491% (30 μ g, $p>0.001$), 826% (100 μ g, $p<0.001$), and 755% (300 μ g, $p<0.001$) of the 8B4 levels. Similarly, the increases in plasma A β x-40 for *Pichia*-derived material were 395% (30 μ g, $p>0.001$), 729% (100 μ g, $p<0.001$), and 838% (300 μ g, $p<0.001$) of the 8B4 levels.

The claims defining the invention are as follows:

1. An isolated antibody or antigen binding fragment thereof capable of differentially recognizing a multi-dimensional conformation of one or more A β -derived diffusible ligands, wherein said antibody or antigen binding fragment comprises a complementary determining region (CDR) of Arg-Xaa₁-Leu-Xaa₂-Xaa₃-Xaa₄-Xaa₅-Xaa₆-Asp-Ala-Met-Asp-Tyr (SEQ ID NO:9), wherein Xaa₁ is Gln or Ala; Xaa₂ is Ser or Gly; Xaa₃ is Pro, Ala, Lys, Arg, or Thr; Xaa₄ is Lys or Arg; Xaa₅ is Gly, Ser, or Lys; Xaa₆ is Val, Thr, Ile or Arg.

2. The isolated antibody or antigen binding fragment of claim 1 comprising:

(a) a light chain variable region comprising,

(i) a CDR1 of SEQ ID NO:154,

(ii) a CDR2 of SEQ ID NO:155, and

(iii) a CDR3 of SEQ ID NO:80; and

(b) a heavy chain variable region comprising,

(i) a CDR1 of SEQ ID NO:156,

(ii) a CDR2 of SEQ ID NO:157, and

(iii) a CDR3 of SEQ ID NO:3.

3. The isolated antibody of claim 1, wherein the heavy and light chain variable regions of said antibody respectively comprise the amino acid sequence set forth in SEQ ID NO:108 and SEQ ID NO:112.

4. The isolated antibody of claim 1, wherein the heavy and light chain of said antibody respectively comprise the amino acid sequence set forth in SEQ ID NO:138 and SEQ ID NO:140.

5. The isolated antibody or antigen binding fragment of claim 1, further comprising a heavy chain constant region of SEQ ID NO:137.

6. The isolated antibody or antigen binding fragment of claim 1, wherein the antibody is a monoclonal antibody.

7. A pharmaceutical composition comprising the antibody or antigen binding fragment of claim 1 in admixture with a pharmaceutically acceptable carrier.

8. A method for attenuating binding of A β -derived diffusible ligands to a neuron comprising contacting the neuron with the antibody or antigen binding fragment of claim 1 so that binding of A β -derived diffusible ligands to the neuron is attenuated.

9. A method for inhibiting assembly of A β -derived diffusible ligands comprising contacting a sample containing amyloid β 1-42 peptides with the antibody or antigen binding fragment of claim 1 thereby inhibiting assembly of A β -derived diffusible ligands.

10. A method for inhibiting the phosphorylation of tau protein at Ser202/Thr205 comprising contacting a sample containing a tau protein with the antibody or antigen binding fragment of claim 1 thereby inhibiting the phosphorylation of tau protein at Ser202/Thr205.

11. A method for attenuating the symptoms of a disease associated with A β -derived diffusible ligands comprising administering an effective amount of the pharmaceutical composition of claim 4.

12. A method for identifying a putative therapeutic agent that attenuates the binding of A β -derived diffusible ligands to neurons comprising

(a) contacting a composition comprising a neuron with A β -derived diffusible ligands in the presence of an agent;

(b) contacting the composition with the antibody or antigen binding fragment of claim 1; and

(c) detecting the amount of antibody or antigen binding fragment bound to the neuron in the presence of the agent,

wherein a decrease in the amount of antibody or antigen binding fragment bound in the presence of the agent as compared to the amount of antibody bound in the

absence of the agent indicates that the agent is a putative therapeutic agent for attenuating binding of A β -derived diffusible ligands to neurons.

13. A method for detecting A β -derived diffusible ligands in a sample comprising contacting the sample with the antibody or antigen binding fragment of claim 1 and determining the presence of a complex comprising the A β -derived diffusible ligands and antibody or antigen binding fragment.

14. A method for diagnosing a disease associated with A β -derived diffusible ligands comprising contacting a biological sample with the antibody or antigen binding fragment of claim 1 and determining the presence of a complex comprising the A β -derived diffusible ligands and the antibody or antigen binding fragment wherein the presence of the complex is diagnostic of a disease associated with A β -derived diffusible ligands.

15. A kit for detecting A β -derived diffusible ligands comprising the antibody or antigen binding fragment of claim 1.

2006306553 02 Jul 2010

1/16

20C2 - HEAVY CHAIN VARIABLE REGION SEQUENCE

tgggcagacttaccattctcattcctgctgctgattgtccctgcatatg
tcttgtccCAAGTTACTCTAAAAGAGTCTGGCCCTGGGATATTGAAGCC
CTCACAGACCCCTCAGTCTGACTTGTTCTCTCTCTGGGTTTTCACTGAGC
ACTTCTGGTATGGGTGTAGGCTGGTTTTCGTCAGCCTTCAGGGAAGGGTC
TGGAGTGGCTGGCACACATTTGGTGGGATGATGATAAGTCCTATAATCC
ATCCCTGAAGAGCCGGCTCACAATCTCCAAGTATACCTCTAGAAACCAG
GTTTTCTCAGATCACCAGTGTGGACACTGCAGATACTGCCACTTACT
ATTGTGCTCGAAGACAACCTCGGACTAAGATCAATTGATGCTATGGACTA
CTGGGGTCAAGGAACCTCAGTCACCGTCTCCTCAGCCAAAACGACACCC
CCATCTGTCTATCCACTG (SEQ ID NO:103)

FIG. 1A

20C2 - LIGHT CHAIN VARIABLE REGION SEQUENCE

agattgcctgtaggctggttgctgatgttctggattcctgcttcca
ccagtGATGTTTTGATGACCCAAACTCCTCTCTCCCTGCCTGTCAGTCT
TGGAGATCAAGCCTCCATCTCTTGCAGATCTAGTCAGAGCATTTCTACAT
AGTAATGGAAACACCTATTTAGAGTGGTACCTGCAGAAACCAGGCCAGT
CTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATTTTCTGGGGTCCC
AGACAGGTTCAAGTGGCAGTGGATCAGGGACAGATTTCACTCAAGATC
AGCAGAGTGGAGGCTGAGGATCTGGGAGTTTATTACTGTTTTCAAGGTT
CACTTGTTCCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGAAACG
GGCTGATGCTGCACCAACTGTATCCATCTTCCCACCATCCAGT
(SEQ ID NO:104)

FIG. 1B

[illegible]

20C2	<u>QLGLRSIDAMDYW</u>	GQGT S VTVSS	(SEQ ID NO:105)
(JH6)	YYYYYGMDVW	GQGT T TVSS	(SEQ ID NO:106)
Hu20C2	<u>QLGLRSIDAMDYW</u>	GQGT T TVSS	(SEQ ID NO:107)
Hu20C2A3	<u>QLGTRGTDAMDYW</u>	GQGT T TVSS	(SEQ ID NO:108)
	HCDR3		

FIG. 2A

VKII 4-1-(1)A18 Hu20C2 Hu20C2A3	20C2	DVLMTQTPLS	LPVSLGDPAS	ISCRSSQSIL	HSNGNTYLEW	YLQKPGQSPK
	(1)A18	DIVMTQTPLS	LSVTPGQPAS	ISCKSSQSLL	HSDGKTYLYW	YLQKPGQSPQ
		DVMTQSPS	LPVTPGEPAS	ISCRSSQSIL	HSNGNTYLEW	YLQKPGQSPQ
		DVMTQTPLS	LPVTPGQPAS	ISCRSSQSIL	HSNGNTYLEW	YLQKPGQSPQ
LCDR1						
VKII 4-1-(1)A18 Hu20C2 Hu20C2A3	20C2	LLIYKVS	SGVPDRFSGS	SGTDFTLKI	SRVEAEDLGV	YYCFQGSLLVP
	(1)A18	LLIYEVSSRF	SGVPDRFSGS	SGTDFTLKI	SRVEAEDVGV	YYCMQGIHLP
		LLIYKVS	SGVPDRFSGS	SGTDFTLKI	SRVEAEDVGV	YYCLQTTTRVP
		LLIYKVS	SGVPDRFSGS	SGTDFTLKI	SRVEAEDVGV	YYCLQTTTRVP
LCDR2						
101						
	20C2	LTFGAGTKLE	LK (SEQ ID NO:109)			
	(JK2)	YTFGQGTKLE	IK (SEQ ID NO:110)			
		LTFGQGTKLE	IK (SEQ ID NO:111)			
		LTFGQGTKLE	IK (SEQ ID NO:112)			

FIG. 2B

4/16

HUMANIZED Hu20C2 (CDR GRAFTED) - HCVRA

caggtgaccctgaaggagtctggccctgccctggtgaagcccacccaga
ccctgaccctgacctgcaccttctctggcttcagcctgagcacctctgg
catgggcgctgggctggatccggcagccccctggcaaggccctggagtgg
ctggcccacatctggtgggacgacgacaagtcctacaaccccagcctga
agagccggctgaccatcagcaaggacaccagcaagaaccaggtggtgct
gaccatgaccaacatggaccctgtggacacagccacctactactgtgcc
cggcggcagctgggcctgcggagcattgatgccatggactactggggcc
agggcaccacagtgacagtgtccagc (SEQ ID NO:113)

FIG. 3A

HUMANIZED Hu20C2 (CDR GRAFTED) - HCVRB

caggtgaccctgaaggagtctggccctgccctggtgaagcccacccaga
ccctgaccctgacctgcacctgtctggcttcagcctgagcacctctgg
catgggcgctgggctggatccggcagccccctggcaaggccctggagtgg
ctggcccacatctggtgggacgacgacaagtcctacaaccccagcctga
agagccggctgaccatcagcaaggacaccagcaagaaccaggtggtgct
gaccatgaccaacatggaccctgtggacacagccacctactactgtgcc
cggcggcagctgggcctgcggagcattgatgccatggactactggggcc
agggcaccacagtgacagtgtccagc (SEQ ID NO:114)

FIG. 3B

HUMANIZED Hu20C2 (CDR GRAFTED) - LCVR

gatgtggtgatgaccagagccccctgtccctgcctgtgaccctggcg
agcctgccagcatctcctgccggagctcccagagcatcctgcactcaa
tggcaacacctacctggagtggtaacctgcagaagcctggccagagcccc
cagctgctgatctacaagggtgtccaaccggttctccggcgtgcctgacc
ggttcagcggctccggcagcggcacagacttcaccctgaagatcagccg
ggtggaggctgaggatgtgggctctactactgcttccagggcagcctg
gtgccctgacctttggccagggcaccaagctggagatcaag (SEQ
ID NO:115)

FIG. 3C

5/16

pFab4 Hu20C2 HEAVY CHAIN VERSION A

MKKTATAIAVALAGFATVAQAALEQVTLKESGPALVKPTQTLTLTCTFS
ompA secretion signal Hu20C2

GFSLSTSGMGVGWIRQPPGKALEWLAHIWWDDDKSYNPSLKSRLTISKD
CDR1 CDR2

TSKNQVVLMTNMDPVDTATYYCARRQLGLRSIDAMDYWGQGTTVTVSS
CDR3

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSG
VHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCTSGHHHHHHGGEQKLISEEDLGG*PFVCEYQGQSSDLPQPPVNA
hCH1 HIS-TAG MYC-TAG ♦Amb

GGGSGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSGSGDFDYEKMANA
pIII stump

NKGAMTENADENALQSDAKGKLDSVATDYGAAIDGFIGDVSGLANGNGA
TGDFAGSNSQMAQVGDGDN SPLMNNFRQYLPSLPQSVECRPYVFGAGKP
YEFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES* (SEQ
ID NO:116)

FIG. 4A

6/16

pFab4 Hu20C2 HEAVY CHAIN VERSION BMKKTAIAIAVALAGFATVAQAALEQVTLKESGPALVKPTQTLTLTCTLS

ompA secretion signal Hu20C2

GFSLSTSGMGVGWIRQPPGKALEWLAHIWWDDDKSYNPSLKSRLTISKD
 CDR1 CDR2

TSKNQVVLMTNMDPVDTATYYCARRQLGLRSIDAMDYWGQGTTVTVSA
 CDR3

STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV
HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCTSGHHHHHHGGEQKLISEEDLGG*PFVCEYQGQSSDLPQPPVNAG
 hCH1 HIS-TAG MYC-TAG ♦Amb

GGSGGGSGGGSEGGGSEGGGSEGGGSEGGGSGGGSGSGDFDYEKMANAN
 pIII stump

KGAMTENADENALQSDAKGKLDVATDYGAAIDGFIGDVSGLANGNGAT
 GDFAGSNSQMAQVGDGDN SPLMNNFRQYLP SLPQSVECRPYVFGAGKPY
 EFSIDCDKINLFRGVFAFLLYVATFMYVFSTFANILRNKES* (SEQ
 ID NO:117)

FIG. 4B**pFab4 Hu20C2 LIGHT CHAIN**MKYLLPTAAAGLLLLLAAQPAMASRDVVMTQSPLSLPVTGPGEPAISCRS

Pel secretion signal Hu20C2

SQSILHSNGNTYLEWYLQKPGQSPQLLIYKVSNRFSGVPDRFSGSGSGT
 CDR1 CDR2

DFTLKISRVEAEDVGVYYCFQGSLVPLTFGQGTKLEIKRTVAAPSVFIF
 CDR3

PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQD
SKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
 hCK (SEQ ID NO:118)

FIG. 4C

7/16

pFab4 Hu20C2 HEAVY CHAIN VERSION A/LIGHT CHAIN

GCCCAATACGCAAACCGCCTCTCCCCGCGCGTTGGCCGATTTCATTAATGCAGCTGGC
ACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTAATGTGAGTT
AGCTCACTCATTAGGCACCCAGGCTTTACACTTTATGCTTCCGGCTCGTATGTTGT
GTGGAATTGTGAGCGGATAACAATTTACACAAGATCTAGCTATTCCAGAGATTACG
CAAATTCATTTCAAGGAGACAGTCATAATGAAATACCTGCTGCCGACTGCAGCTGC
TGGTCTGCTGCTGCTGGCGGCCAGCCGGCTATGGCTTCTAGAGatgtggtgatgac
ccagagccccctgtccctgcctgtgacccctggcgagcctgccagcatctcctgccg
gagctcccagagcatcctgcactccaatggcaacacctacctggagtggtacctgca
gaagcctggccagagccccagctgctgatctacaagggtgtccaaccgggtctccgg
cgtgcctgaccgggttcagcggtcccgccagcgccacagacttcaccctgaagatcag
ccgggtggaggctgaggatgtgggcgtctactactgcttccagggcagcctggtgcc
cctgacctttggccagggcaccaagctggagatcaagCGTACGGTGGCTGCACCATC
TGTCTTCATCTTCCCGCCATCTGATGAGCAGTTGAAATCTGGAAGTGCCTCTGTTGT
GTGCCGTGCTGAATAACTTCTATCCCAGAGAGGCCAAAGTACAGTGGAAGGTGGATAA
CGCCCTCCAATCGGGTAACTCCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAG
CACCTACAGCCTCAGCAGCACCTGACGCTGAGCAAAGCAGACTACGAGAAACACAA
AGTCTACGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCGTCACAAAGAGCTT
CAACAGGGGAGAGTGTTAAACAATTGCTAGAAATTGTGAGCGGATAACAATTTAGCAG
GTCGAGTTCTTGATAACGAGGCGTAAAAATGAAAAAGACAGCTATCGCGATTGCAG
TGGCACTGGCTGGTTTTCGCTACCGTGGCCAGGCGGCCCTCGAGcaggtgacctga
aggagtctggccctgccctggtgaagcccaccagacctgacctgacctgacct
tctctggcttcagcctgagcacctctggcatgggcgtgggctggatccggcagcccc
ctggcaaggccctggagtggctggccacatctggtgggacgacgacaagtcttaca
accccagcctgaagagccggctgacctcagcaaggacaccagcaagaaccagggtgg
tgctgacctgaccaacatggacctgtggacacagccacctactactgtgcccggc
ggcagctgggcctgaggagcattgatgccatggactactggggccaggggcaccacag
tgacagtgtccagcGCCTCCACCAAGGGCCCATCGGTCTTCCCCCTGGCACCTCCT
CCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCC
CCGAACCGGTGACGGTGTCTGTGGAAGTACAGCGCCCTGACCAGCGGCGTGCACACCT
TCCCGGCTGTCTACAGTCTCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGC
CCTCCAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAGCCCAGCA
ACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGTACTAGTGGCCACCACCACC
ATCACCATGGCGGTGAACAAAACTCATCTCAGAAGAGGATCTGGGTGGTTAGCCAT
TCGTTTGTGAATATCAAGGCCAATCGTCTGACCTGCCTCAACCTCCTGTCAATGCTG
GCGGCGGCTCTGGTGGTGGTTCTGGTGGCGGCTCTGAGGGTGGCGGCTCTGAGGGTG
GCGGTTCTGAGGGTGGCGGCTCTGAGGGTGGCGGTTCCGGTGGCGGCTCCGGTTCCG
GTGATTTTGATTATGAAAAATGGCAAACGCTAATAAGGGGGCTATGACCGAAAATG
CCGATGAAAACGCGCTACAGTCTGACGCTAAAGGCAAACCTTGATTCTGTGCTACTG
ATTACGGTGTCTATCGATGGTTTCATTGGTGACGTTTCCGGCCTTGCTAATGGTA
ATGGTGCTACTGGTGATTTTGTGCTGGCTCTAATTCCTCAATGGCTCAAGTCGGTGACG
GTGATAATTCACCTTTAATGAATAATTTCCGTCAATATTTACCTTCTTTGCCTCAGT
CGGTTGAATGTGCCCCCTTATGTCTTTGGCGCTGGTAAACCATATGAATTTTCTATTG
ATTGTGACAAAATAAACTTATTCCGTGGTGTCTTTGCGTTTCTTTTATATGTTGCCA
CCTTTATGTATGTATTTTCGACGTTTGCTAACATACTGCGTAATAAGGAGTCTTAAG
CTAGCTGGCCGTCGTTTTACAACGTCGTGACTGGGAAAACCTGGCGTTACCCAACT
TAATCGCCTTGAGCACATCCCCCTTTCGCCAGCTGGCGTAATAGCGAAGAGGCCCG
CACCGATCGCCCTTCCCAACAGTTGCGCAGCCTGAATGGCGAATGGCGATTCCGTTG

FIG. 4D

8/16

CAATGGCTGGCGGTAATATTGTTCTGGATATTACCAGCAAGGCCGATAGTTTGAGTT
CTTCTACTCAGGCAAGTGATGTTATTACTAATCAAAGAAGTATTGCGACAACGGTTA
ATTTGCGTGATGGACAGACTCTTTTACTCGGTGGCCTCACTGATTATAAAAACACTT
CTCAGGATTCTGGCGTACCGTTCTCTGTCTAAAATCCCTTTAATCGGCCTCCTGTTTA
GCTCCCGCTCTGATTCTAACGAGGAAAGCACGTTATACGTGCTCGTCAAAGCAACCA
TAGTACGCGCCCTGTAGCGGCGCATTAAGCGCGGCGGGTGTGGTGGTTACGCGCAGC
GTGACCGCTACACTTGCCAGCGCCCTAGCGCCCGCTCCTTTTCGCTTTCTCCCTTCC
TTTCTCGCCACGTTTCGCCGGCTTTCCCCGTCAAGCTCTAAATCGGGGGCTCCCTTTA
GGGTTCCGATTTAGTGCTTTACGGCACCTCGACCCCAAAAACTTGATTAGGGTGAT
GGTTCACGTAGTGGGCCATCGCCCTGATAGACGGTTTTTCGCCCTTTGACGTTGGAG
TCCACGTTCTTTAATAGTGGACTCTTGTTCCAACTGGAACAACACTCAACCCTATC
TCGGTCTATTCTTTTGATTTATAAGGGATTTTGCCGATTTTCGGCCTATTGGTTAAAA
AATGAGCTGATTTAACAAAAATTTAACGCGAATTTTAACAAAATATTAACGTTTACA
ATTTAAATATTTGCTTATACAATCTTCCTGTTTTTGGGGCTTTTCTGATTATCAACC
GGGTACATATGATTGACATGCTAGTTTTACGATTACCGTTCATCGCAGGTGGCACT
TTTCGGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTTCTAAATACATTCAAAAT
ATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTGAAAAAGG
AAGAGTATGAGTATTCACATTTCCGTGTGCCCCCTATTCCCTTTTTTGCGGCATTT
TGCCTTCCTGTTTTTGCTCACCCAGAAACGCTGGTGAAAGTAAAGATGCTGAAGAT
CAGTTGGGTGCACGAGTGGGTTACATCGAACTGGATCTCAACAGCGGTAAGATCCTT
GAGAGTTTTTCGCCCGAAGAACGTTTTCCAATGATGAGCACTTTTAAAGTTCTGCTA
TGTGGCGCGGTATTATCCCGTATTGACGCCGGGCAAGAGCAACTCGGTGCGCCGATA
CACTATTCTCAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACG
GATGGCATGACAGTAAGAGAATTATGCAGTGCTGCCATAACCATGAGTGATAACACT
GCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCCTTTTTTG
CACAACATGGGGGATCATGTAACCTCGCCTTGATCGTTGGGAACCGGAGCTGAATGAA
GCCATACCAAACGACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTG
CGAAACTATTAACCTGGCGAACTACTTACTCTAGCTTCCCGGCAACAATTAATAGAC
TGGATGGAGGCGGATAAAGTTGCAGGACCCTTCTGCGCTCGGCCCTTCCGGCTGGC
TGGTTTATTGCTGATAAATCTGGAGCCGGTGAGCGTGGGTCTCGCGGTATCATTGCA
GCACTGGGGCCAGATGGTAAGCCCTCCCGTATCGTAGTTATCTACACGACGGGAGT
CAGGCAACTATGGATGAACGAAATAGACAGATCGCTGAGATAGGTGCCTCACTGATT
AAGCATTGGTAACTGTGACACCAAGTTTACTCATATATACTTTAGATTGATTAAAA
CTTCATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTTGATAATCTCATGACC
AAAATCCCTTAACGTGAGTTTTTCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATC
AAAGGATCTTCTTGAGATCCTTTTTTTCTGCGCGTAATCTGCTGCTTGCAAAACAAA
AAACCACCGCTACCAGCGGTGGTTTTGTTTGGCGGATCAAGAGCTACCAACTCTTTTT
CCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAATACTGTCTTCTAGTGATG
CTAATCCTGTACCAGTGCTGCTGCTGCCAGTGCGGATAAGTCGTGTCTTACCGGGTTG
GACTCAAGACGATAGTTACCGGATAAGGCGCAGCGGTGGGGCTGAACGGGGGGTTG
TGCACACAGCCAGCTTGGAGCGAACGACCTACACCGAACTGAGATACCTACAGCGT
GAGCTATGAGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTA
AGCGGCAGGGTTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGG
TATCTTTATAGTCCTGTGCGGTTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGA
TGCTCGTCAGGGGGGCGGAGCCTATGGAAAAACGCCAGCAACGCGGCCTTTTTACGG
TTCTGGCCTTTTGCTGGCCTTTTGCTCACATGTTCTTCTGCGTTATCCCTGAT
TCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCTGATACCGCTCGCCGCAGCCGA
ACGACCGAGCGCAGCGAGTCAGTGAGCGAGGAAGCGGAAGAGC (SEQ ID
NO: 119)

FIG. 4E

Biotin-5'-cagccaccgtacgCTTGATCTCCAGCTTGGTGCCTGGCCAAAMNNMNMNMMNNGTCGCCCTGGAAGCAGTAGTAGAC 82
(Primer 20C2LC3-2; SEQ ID NO:125)

FIG. 5A

10/16

FIG. 5B

11/16

```

      |--- CH1 STARTS HERE          C144
IgG1  /// ASTK GPSVFPLAPS SKSTSGGTAA LGCLVKDYFP EPVTVSWNSG
IgG2  /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
IgG4  /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
IgG2M4 /// ASTK GPSVFPLAPC SRSTSESTAA LGCLVKDYFP EPVTVSWNSG
      (VH-C1 LINKER)

                                          C200
IgG1  ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTQTYICN VNHKPSNTKV
IgG2  ALTSGVHTFP AVLQSSGLYS LSSVVTVTSS NFGTQTYTCN VDHKPSNTKV
IgG4  ALTSGVHTFP AVLQSSGLYS LSSVVTVPSS SLGTKTYTCN VDHKPSNTKV
IgG2M4 ALTSGVHTFP AVLQSSGLYS LSSVVTVTSS NFGTQTYTCN VDHKPSNTKV

      -HINGE REGION--||---CH2-> P238          M252          C261
IgG1  DKKAEPKSCD KTHTCPPCPA PELLGGPSVF LFPPKPKDTL MISRTPEVTC
IgG2  DKTVERKCC- --VECPPCPA PP-VAGPSVF LFPPKPKDTL MISRTPEVTC
IgG4  DKRVESKYGP ---PCPSCPA PEFLGGPSVF LFPPKPKDTL MISRTPEVTC
IgG2M4 DKTVERKCC- --VECPPCPA PP-VAGPSVF LFPPKPKDTL MISRTPEVTC
      (LOWER HINGE)          FcRn-BIND

      Q268          N297*          L309
IgG1  VVVDVSHEDP EVKFNWYVDG VEVHNAKTKP REEQYNSTYR VVSVLTIVLHQ
IgG2  VVVDVSHEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTIVVHQ
IgG4  VVVDVSOQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTYR VVSVLTIVLHQ
IgG2M4 VVVDVSOQEDP EVQFNWYVDG VEVHNAKTKP REEQFNSTFR VVSVLTIVLHQ
      B/C LOOP          C'E LOOP          FcRn-BIND

      P331
      C321          A330          |---CH3->
IgG1  DWLNGKEYKC KVSNAKLPAPI EKTISKAKG QPREPQVYTL PPSRDELTKN
IgG2  DWLNGKEYKC KVSNAKGLPAPI EKTISKTKG QPREPQVYTL PPSREEMTKN
IgG4  DWLNGKEYKC KVSNAKGLPSSI EKTISKAKG QPREPQVYTL PPSQEEMTKN
IgG2M4 DWLNGKEYKC KVSNAKGLPSSI EKTISKTKG QPREPQVYTL PPSREEMTKN
      F/G LOOP

IgG1  QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSKLT
IgG2  QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPMLDSD GSFFLYSKLT
IgG4  QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPVLDSD GSFFLYSRLT
IgG2M4 QVSLTCLVKG FYPSDIAVEW ESNGQPENNY KTTTPMLDSD GSFFLYSKLT

      M428L H433
IgG1  VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:134)
IgG2  VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:135)
IgG4  VDKSRWQEGN VFSCSVMHEA LHNHYTQKSL SLSLGK* (SEQ ID NO:136)
IgG2M4 VDKSRWQQGN VFSCSVMHEA LHNHYTQKSL SLSPGK* (SEQ ID NO:137)
      FcRn-BIND

```

FIG. 6

12/16

HUMANIZED Hu20C2A3 IgG2M4 - HEAVY CHAIN

1 QVTLKESGPG LLKPTQTLTL TCTLSGFSLT TSGMGVGVWFR QPPGKGLEWL
51 AHIWWDDDKS YNPSLKSRLLT ISKDTSKNQV VLTITNVDPV DTATYYCARR
101 QLGTRGTDAM DYWGQGTTVT VSSASTKGPS VFPLAPCSRS TSESTAALGC
151 LVKDYFPEPV TVSWNSGALT SGVHTFPAVL QSSGLYSLSS VVTVTSSNFG
201 TQTYTCNVDH KPSNTKVDKT VERKCCVECP PCPAPPVAGP SVFLFPPKPK
251 DTLMISRTPE VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS
301 TFRVSVSLTV LHQDWLNGKE YKCKVSNKGL PSSIEKTISK TKGQPREPQV
351 YTLPPSREEM TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPML
401 DSDGSFFLYS KLTVDKSRWQ QGNVFSCSVM HEALHNHYTQ KSLSLSPGK
(SEQ ID NO:138)

FIG. 7A

HUMANIZED Hu20C2A3 IgG2M4 - HEAVY CHAIN

ATGGAATGGAGCTGGGTCTTTCTCTTCTCCTGTCAGTAACTACAGGTGT
CCACTCGCAGGTGACCCTGAAGGAGTCTGGCCCTGGCCTGCTGAAGCCCA
CCCAGACCCTGACCCTGACCCTGCACCCTGTCTGGCTTCAGCCTGAGCACC
TCTGGCATGGGCGTGGGCTGGTTCGGGCAGCCCCCTGGCAAGGGCCTGGA
GTGGCTGGCCCACATCTGGTGGGACGACGACAAGTCCTACAACCCCAGCC
TGAAGAGCCGGCTGACCATCAGCAAGGACACCAGCAAGAACCAGGTGGTG
CTGACCATCACCAACGTGGACCCTGTGGACACAGCCACCTACTACTGTGC
CCGGCGGCAGCTGGGCACTAGGGGGGACGGATGCCATGGACTACTGGGGCC
AGGGCACCCACAGTGACAGTGTCCAGCGCATCCACCAAGGGCCCATCCGTC
TTCCCCCTGGCGCCCTGCTCCAGGAGCACCTCCGAGAGCACAGCCGCCCT
GGGCTGCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGA
ACTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTACAG
TCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGACCTCCAGCAA
CTTTGGCACGCAGACCTACACCTGCAACGTAGATCACAAGCCCAGCAACA
CCAAGGTGGACAAGACAGTTGAGCGGAAATGCTGCGTGGAGTGCCACCA
TGCCCAGCACCTCCAGTGGCCGGACCATCAGTCTTCCTGTTCCCCCCTAA
ACCCAAGGACACTCTCATGATCTCCCGGACCCCTGAGGTACAGTGCGTGG
TGGTGGACGTGAGCCAGGAAGACCCCGAGGTCCAGTTCAACTGGTACGTG
GATGGCGTGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTT
CAACAGCACGTTCCGTGTGGTCAGCGTCCTCACCGTCCTGCACCAGGACT
GGCTGAACGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGGCCTCCCG
TCCTCCATCGAGAAAACCATCTCCAAAACCAAGGGCAGCCCCGAGAGCC
ACAGGTGTACACCCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGG
TCAGCCTGACCTGCCTGGTCAAAGGCTTCTACCCAGCGACATCGCCGTG
GAGTGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCC
CATGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTAACCGTGG
ACAAGAGCAGGTGGCAGCAGGGGAATGTCTTCTCATGCTCCGTGATGCAT
GAGGCTCTGCACAACCACTACACACAGAAGAGCCTCTCCCTGTCTCTCTGG
TAAATGA (SEQ ID NO:139)

FIG. 7B

13/16

HUMANIZED Hu20C2A3 - LIGHT CHAIN

1 DVVMTQTPLS LPVTPGQPAS ISCRSSQSIL HSNGNTYLEW YLQKPGQSPQ
51 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCLQTTRVP
101 LTFGQGTKLE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
151 VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
201 VTHQGLSSPV TKSFNRGEC (SEQ ID NO:140)

FIG. 7C

HUMANIZED Hu20C2A3 - LIGHT CHAIN

ATGAGTGTGCCCACTCAGGTCCTGGGGTTGCTGCTGCTGTGGCTTACAGA
TGCCAGATGCGATGTGGTGATGACCCAGACCCCCCTGTCCCTGCCTGTGA
CCCCTGGCCAGCCTGCCAGCATCTCCTGCCGGAGCTCCAGAGCATCCTG
CACTCCAATGGCAACACCTACCTGGAGTGGTACCTGCAGAAGCCTGGCCA
GAGCCCCCAGCTGCTGATCTACAAGGTGTCCAACCGGTTCTCCGGCGTG
CTGACCGGTTTCAGCGGCTCCGGCAGCGGCACAGACTTCACCCTGAAGATC
AGCCGGGTGGAGGCTGAGGATGTGGGCGTCTACTACTGCCTTCAGACTAC
TCGTGTGCCCCCTGACCTTTGGCCAGGGCACCAAGCTGGAGATCAAGCGTA
CGGTGGCTGCACCATCTGTCTTCATCTTCCCGCCATCTGATGAGCAGTTG
AAATCTGGAAGTGCCTCTGTTGTGTGCCTGCTGAATAACTTCTATCCCAG
AGAGGCCAAAGTACAGTGGAAGGTGGATAACGCCCTCCAATCGGGTAACT
CCCAGGAGAGTGTACAGAGCAGGACAGCAAGGACAGCACCTACAGCCTC
AGCAGCACCCCTGACGCTGAGCAAAGCAGACTACGAGAAACACAAAGTCTA
CGCCTGCGAAGTCACCCATCAGGGCCTGAGCTCGCCCGTCACAAAGAGCT
TCAACAGGGGAGAGTGTTAG (SEQ ID NO:141)

FIG. 7D

14/16

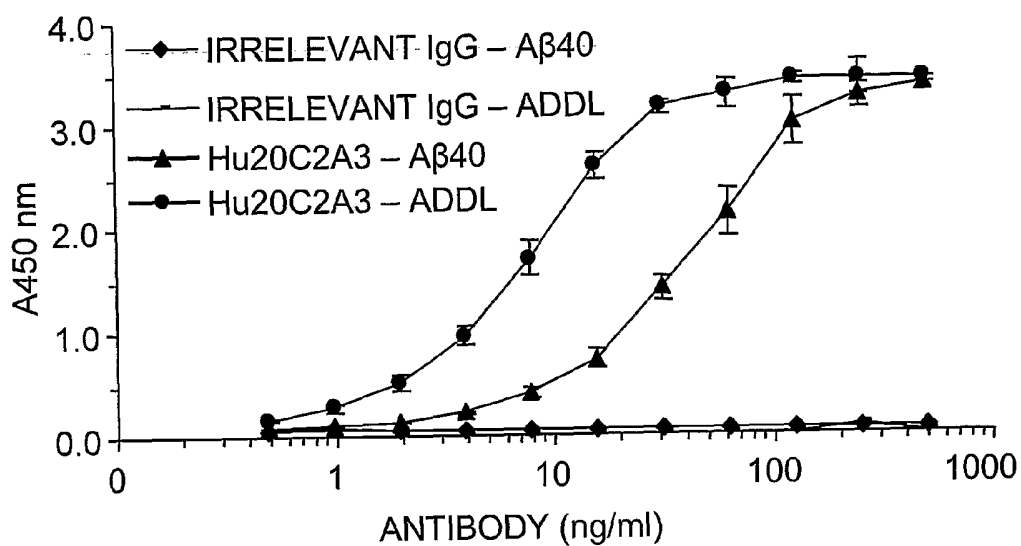


FIG. 8A

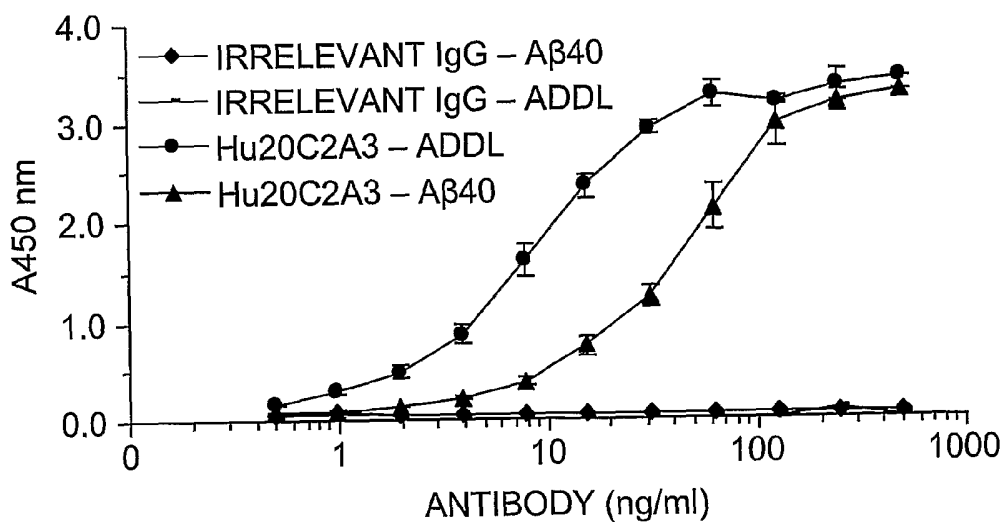
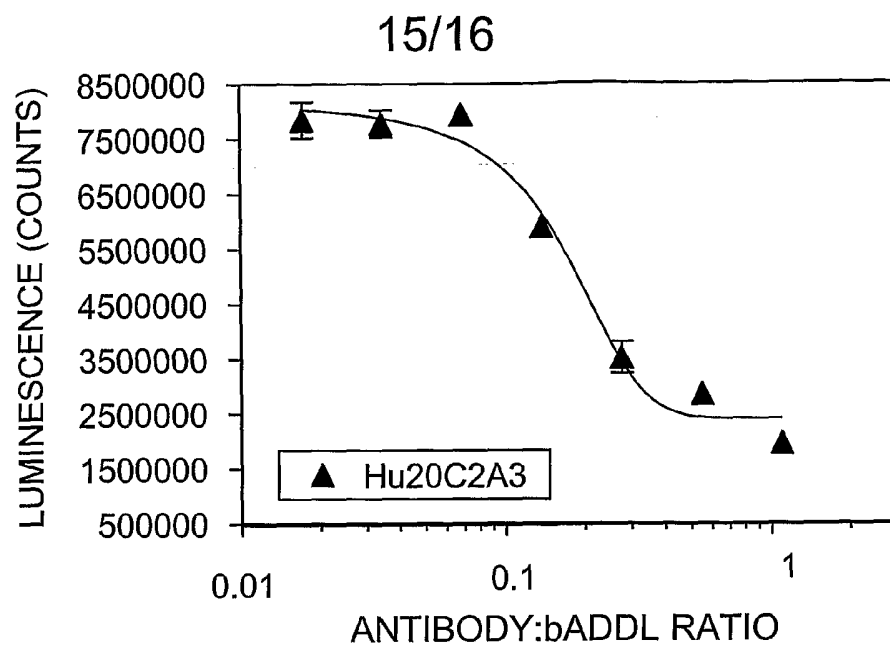
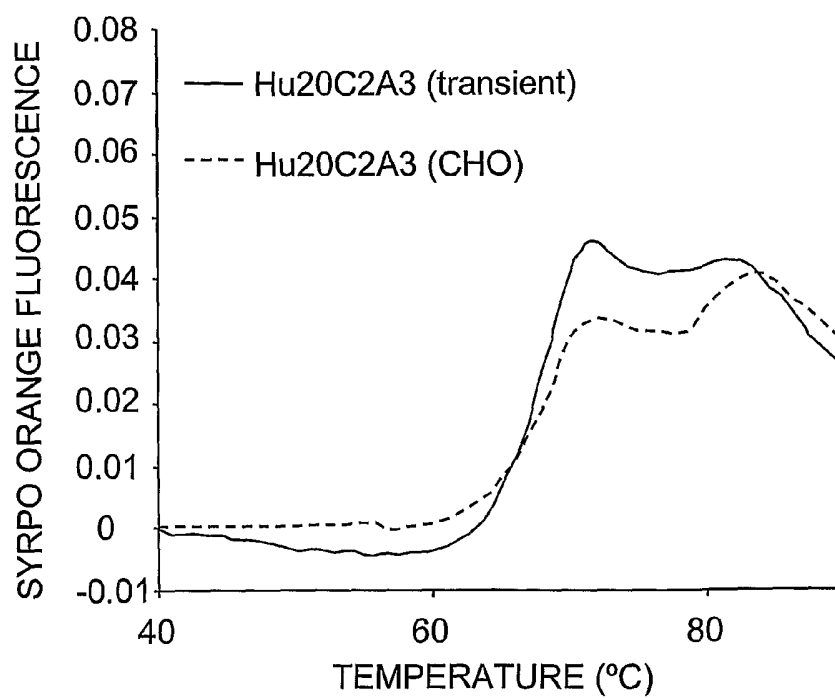


FIG. 8B

*FIG. 9**FIG. 10*

16/16

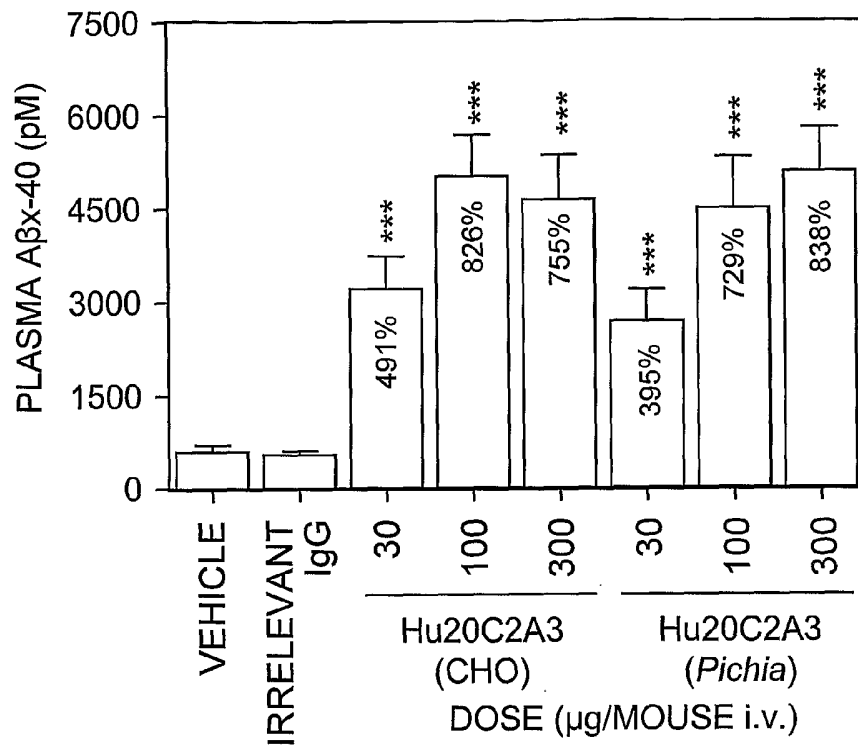


FIG. 11

SEQUENCE LISTING

<110> Kinney, Gene
Strohl, William R.
An, Zhiqiang

<120> ANTI-ADDL MONOCLONAL ANTIBODY AND USE THEREOF

<130> MRK0003WO

<150> PCT/US2005/038125

<151> 2005-10-21

<160> 153

<170> PatentIn version 3.3

<210> 1

<211> 6

<212> PRT

<213> Homo sapiens

<400> 1

Glu Phe Arg His Asp Ser
1 5

<210> 2

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<220>

<221> MISC_FEATURE

<222> (1)..(1)

<223> Xaa denotes Phe or Leu

<220>

<221> MISC_FEATURE

<222> (3)..(3)

<223> Xaa denotes Ala or Thr

<400> 2

Xaa Gln Xaa Thr Arg Val Pro Leu Thr
1 5

<210> 3

<211> 13

<212> PRT

<213> Artificial Sequence

- 2 -

<220>

<223> Synthetic CDR3 peptide

<400> 3

Arg Gln Leu Gly Thr Arg Gly Thr Asp Ala Met Asp Tyr
1 5 10

<210> 4

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<400> 4

Arg Ala Leu Ser Pro Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 5

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<400> 5

Arg Gln Leu Gly Ala Arg Lys Thr Asp Ala Met Asp Tyr
1 5 10

<210> 6

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<400> 6

Arg Gln Leu Gly Pro Arg Lys Arg Asp Ala Met Asp Tyr
1 5 10

<210> 7

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

- 3 -

<400> 7

Arg Gln Leu Gly Lys Leu Lys Thr Asp Ala Met Asp Tyr
1 5 10

<210> 8

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<400> 8

Arg Gln Leu Gly Arg Arg Ser Val Asp Ala Met Asp Tyr
1 5 10

<210> 9

<211> 13

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic CDR3 peptide

<220>

<221> MISC_FEATURE

<222> (2)..(2)

<223> Xaa denotes Gln or Ala

<220>

<221> MISC_FEATURE

<222> (4)..(4)

<223> Xaa denotes Ser or Gly

<220>

<221> MISC_FEATURE

<222> (5)..(5)

<223> Xaa denotes Pro, Ala, Lys, Arg, or Thr

<220>

<221> MISC_FEATURE

<222> (6)..(6)

<223> Xaa denotes Lys or Arg

<220>

<221> MISC_FEATURE

<222> (7)..(7)

<223> Xaa denotes Gly, Ser, or Lys

<220>

<221> MISC_FEATURE

<222> (8)..(8)

<223> Xaa denotes Val, Thr, Ile or Arg

- 4 -

<400> 9

Arg Xaa Leu Xaa Xaa Xaa Xaa Xaa Asp Ala Met Asp Tyr
1 5 10

<210> 10

<211> 10

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 10

Leu Pro Val Thr Pro Gly Glu Pro Ala Ser
1 5 10

<210> 11

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 11

gatctctaga tgaagattgc ctgttaggct gttgggtgctg

40

<210> 12

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 12

gatctctaga tggagwcaga cacactcctg ytatgggtg

39

<210> 13

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 13

gatctctaga tgagtgtgct cactcaggctc ctggsgttg

39

<210> 14

<211> 42

<212> DNA

- 5 -

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 14

gatctctaga tgaggrcccc tgctcagwtt yttggmwtct tg

42

<210> 15

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 15

gatctctaga tggatttwca ggtgcagatt wtcagcttc

39

<210> 16

<211> 38

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 16

gatctctaga tgaggtkcyy tgytsaycty ctctgrgg

38

<210> 17

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 17

gatctctaga tgggcwtcaa agatggagtc acakwyycwg g

41

<210> 18

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 18

gatctctaga tgtggggayc tktttycmmt ttttcaatg

39

<210> 19

<211> 34

- 6 -

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 19
gatctctaga tggtrtccwc asctcagttc cttg 34

<210> 20
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 20
gatctctaga tgtatatatg tttgttgtct atttct 36

<210> 21
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 21
gatctctaga tggaagcccc agctcagctt ctcttcc 37

<210> 22
<211> 30
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 22
gatcgagctc actggatggt gggaagatgg 30

<210> 23
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 23
gatctctaga tgaaatgcag ctggggcats ttcttc 36

<210> 24

- 7 -

<211> 35
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 24
gatctctaga tgggatggag ctrtatcats ytctt 35

<210> 25
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 25
gatctctaga tgaagwtgtg gttaactgg gttttt 36

<210> 26
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 26
gatctctaga tgractttgg gytcagcttg rttt 34

<210> 27
<211> 41
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 27
gatctctaga tgggactcca ggcttcaatt tagttttcct t 41

<210> 28
<211> 38
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 28
gatctctaga tggcttgcty ttrgsgctrc tcttctgc 38

- 8 -

<210> 29
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 29
gatctctaga tggratggag ckggrgtctt tmtctt 36

<210> 30
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 30
gatctctaga tgagagtgcg gattcttttg tg 32

<210> 31
<211> 40
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 31
gatctctaga tggmttgggt gtggamcttg cttattcctg 40

<210> 32
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 32
gatctctaga tgggcagact taccattctc attcctg 37

<210> 33
<211> 37
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 33
gatctctaga tggatttttg gctgattttt tttattg 37

- 9 -

<210> 34
<211> 36
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 34
gatctctaga tgatggtggt aagtcttctg tacctg 36

<210> 35
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 35
gcatcgagct ccagtggata gacagatggg gg 32

<210> 36
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 36
gcatcgagct ccagtggata gaccgatggg gg 32

<210> 37
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 37
gcatcgagct ccagtggatg agctgatggg gg 32

<210> 38
<211> 32
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 38
gcatcgagct ccaagggata gacagatggg gc 32

- 10 -

<210> 39
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 39

Phe Gln Gly Ser Leu Val Pro Leu Thr
1 5

<210> 40
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (1)..(5)
<223> Xaa denotes any amino acid residue

<400> 40

Xaa Xaa Xaa Xaa Xaa Val Pro Leu Thr
1 5

<210> 41
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (5)..(9)
<223> Xaa denotes any amino acid residue

<400> 41

Phe Gln Gly Ser Xaa Xaa Xaa Xaa Xaa
1 5

<210> 42
<211> 9
<212> PRT

- 11 -

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 42

Ala Asp Thr Thr His Val Pro Leu Thr
1 5

<210> 43

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 43

Ala His Ser Thr Phe Val Pro Leu Thr
1 5

<210> 44

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 44

Ala Gln Ala Ser Phe Val Pro Leu Thr
1 5

<210> 45

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 45

Ala Gln Ala Thr Lys Val Pro Leu Thr
1 5

<210> 46

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

- 12 -

<223> Synthetic peptide

<400> 46

Ala Gln Ser Ser Lys Val Pro Leu Thr
1 5

<210> 47

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 47

Ala Gln Ser Thr Leu Val Pro Leu Thr
1 5

<210> 48

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 48

Phe Ala Ala Ser Ser Val Pro Leu Thr
1 5

<210> 49

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 49

Phe Glu Ser Thr Tyr Val Pro Leu Thr
1 5

<210> 50

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 50

- 13 -

Phe Glu Ser Ser Arg Val Pro Leu Thr
1 5

<210> 51
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 51

Phe Asn Ala Thr Trp Val Pro Leu Thr
1 5

<210> 52
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 52

Phe Gln Ala Ser Arg Val Pro Leu Thr
1 5

<210> 53
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 53

Phe Gln Ala Thr Arg Val Pro Leu Thr
1 5

<210> 54
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 54

Phe Gln Gly Ser Phe Ile Gly Leu Ser
1 5

- 14 -

<210> 55
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 55

Phe Gln Gly Ser Phe Ile Pro Gly Thr
1 5

<210> 56
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 56

Phe Gln Gly Ser Phe Leu Pro Pro Ser
1 5

<210> 57
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 57

Phe Gln Gly Ser Phe Leu Pro Gln Leu
1 5

<210> 58
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 58

Phe Gln Gly Ser Leu Phe Pro Pro Val
1 5

<210> 59

- 15 -

<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 59

Phe Gln Gly Ser Leu Phe Ser Pro Ser
1 5

<210> 60
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 60

Phe Gln Gly Ser Arg Ile Pro Ile Ser
1 5

<210> 61
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 61

Phe Gln Gly Ser Arg Leu Pro Val Ser
1 5

<210> 62
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 62

Phe Gln Gly Ser Arg Val Pro Leu Val
1 5

<210> 63
<211> 9
<212> PRT
<213> Artificial Sequence

- 16 -

<220>

<223> Synthetic peptide

<400> 63

Phe Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 64

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 64

Phe Gln Ser Ser Arg Val Pro Leu Thr
1 5

<210> 65

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 65

Gly Gln Thr Thr Leu Val Pro Leu Thr
1 5

<210> 66

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 66

His Glu Ser Thr Leu Val Pro Leu Thr
1 5

<210> 67

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

- 17 -

<400> 67

His Gln Ser Ser Lys Val Pro Leu Thr
1 5

<210> 68

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 68

Ile Gln Thr Ser Leu Val Pro Leu Thr
1 5

<210> 69

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 69

Ile Gln Ala Ala Leu Val Pro Leu Thr
1 5

<210> 70

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 70

Leu Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 71

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 71

- 18 -

Leu Glu Thr Ser Arg Val Pro Leu Thr
1 5

<210> 72
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 72

Leu Ala Ser Ser His Val Pro Leu Thr
1 5

<210> 73
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 73

Leu Asn Ser Thr Thr Val Pro Leu Thr
1 5

<210> 74
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 74

Leu Gln Ser Lys Ser Val Pro Leu Thr
1 5

<210> 75
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 75

Leu Gln Ser Val Arg Val Pro Leu Thr
1 5

- 19 -

<210> 76
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 76

Leu Gln Ser Ser Leu Val Pro Leu Thr
1 5

<210> 77
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 77

Leu Gln Thr Gly Arg Val Pro Leu Thr
1 5

<210> 78
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 78

Leu Gln Thr Ser Phe Val Pro Leu Thr
1 5

<210> 79
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 79

Leu Gln Thr Ser Asn Val Pro Leu Thr
1 5

<210> 80
<211> 9

- 20 -

<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 80

Leu Gln Thr Thr Arg Val Pro Leu Thr
1 5

<210> 81
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 81

Leu Ser Ser Thr Phe Val Pro Leu Thr
1 5

<210> 82
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 82

Leu Ser Ser Thr His Val Pro Leu Thr
1 5

<210> 83
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 83

Leu Thr Ser Ser Ala Val Pro Leu Thr
1 5

<210> 84
<211> 9
<212> PRT
<213> Artificial Sequence

- 21 -

<220>

<223> Synthetic peptide

<400> 84

Leu Val Ser Ser Leu Val Pro Leu Thr
1 5

<210> 85

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 85

Met Glu Thr Ala Asn Val Pro Leu Thr
1 5

<210> 86

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 86

Met Gln Ser Ser Phe Val Pro Leu Thr
1 5

<210> 87

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 87

Met Gln Ser Ser Leu Val Pro Leu Thr
1 5

<210> 88

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

- 22 -

<400> 88

Met Gln Thr Ser Lys Val Pro Leu Thr
1 5

<210> 89

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 89

Ser Gln Ala Arg Met Val Pro Leu Thr
1 5

<210> 90

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 90

Ser Gln Ala Ser Arg Val Pro Leu Thr
1 5

<210> 91

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 91

Thr Gln Ser Thr Gln Val Pro Leu Thr
1 5

<210> 92

<211> 9

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic peptide

<400> 92

Val Cys Ala Thr Phe Val Pro Leu Thr

- 23 -

1 5

<210> 93
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 93

Val Gln Ser Ser Ala Val Pro Leu Thr
1 5

<210> 94
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 94

Val Gln Thr Ser Leu Val Pro Leu Thr
1 5

<210> 95
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 95

Val Gln Thr Ser Val Val Pro Leu Thr
1 5

<210> 96
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 96

Val Gln Thr Thr Ala Val Pro Leu Thr
1 5

- 24 -

<210> 97
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 97

Leu Gln Thr Ala Arg Val Pro Leu Thr
1 5

<210> 98
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 98

Phe Gln Gly Ser Leu Leu Pro Leu Ser
1 5

<210> 99
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 99

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 100
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<220>
<221> MISC_FEATURE
<222> (1)..(5)
<223> Xaa denotes any amino acid residue

<400> 100

Xaa Xaa Xaa Xaa Xaa Arg Ser Ile Asp Ala Met Asp Tyr

1 5 10

```
<400> 103
tgggcagact taccattctc attcctgctg ctgattgtcc ctgcatatgt cttgtcccaa      60
gttactctaa aagagtctgg ccctgggata ttgaagccct cacagaccct cagtctgact      120
tgttctctct ctgggttttc actgagcact tctgggatgg gtgtaggctg gtttcgtcag      180
ccttcaggga agggctctgga gtggctggca cacatttggt gggatgatga taagtccat      240
aatccatccc tgaagagccg gctcacaaac tccaagtata cctctagaaa ccagggttttc      300
```

- 26 -

ctcaccgatca ccagtgtgga cactgcagat actgccactt actattgtgc tcgaagacaa 360
 ctccggactaa gatcaattga tgctatggac tactgggggtc aaggaacctc agtcaccgtc 420
 tcctcagcca aaacgacacc cccatctgtc tatccactg 459

<210> 104
 <211> 435
 <212> DNA
 <213> Mus musculus

<400> 104
 agattgcctg ttaggctgtt ggtgctgatg ttctggattc ctgcttcac cagtgatgtt 60
 ttgatgaccc aaactcctct ctccctgcct gtcagtcttg gagatcaagc ctccatctct 120
 tgcagatcta gtcagagcat totacatagt aatggaaaca cctattttaga gtggtacctg 180
 cagaaaccag gccagtctcc aaagctcctg atctacaaaag tttccaaccg attttctggg 240
 gtcccagaca ggttcagtgg cagtggatca gggacagatt tcacactcaa gatcagcaga 300
 gtggaggctg aggatctggg agtttattac tgttttcaag gttcacttgt tccgctcacg 360
 ttcgggtgctg ggaccaagct ggagctgaaa cgggctgatg ctgcaccaac tgtatccatc 420
 ttcccacat ccagt 435

<210> 105
 <211> 123
 <212> PRT
 <213> Mus musculus

<400> 105

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

Thr Leu Ser Leu Thr Cys Ser Leu Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Ser Gly Lys Gly Leu Glu
 35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Tyr Thr Ser Arg Asn Gln Val
 65 70 75 80

Phe Leu Thr Ile Thr Ser Val Asp Thr Ala Asp Thr Ala Thr Tyr Tyr
 85 90 95

- 27 -

Cys Ala Arg Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
 100 105 110

Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
 115 120

<210> 106
 <211> 120
 <212> PRT
 <213> Homo sapiens

<400> 106

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

Thr Leu Thr Leu Thr Cys Thr Phe Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

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

Trp Leu Ala Arg Ile Asp Trp Asp Asp Asp Lys Phe Tyr Ser Thr Ser
 50 55 60

Leu Lys Thr Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

Cys Ala Arg Ile Tyr Tyr Tyr Tyr Tyr Gly Met Asp Val Trp Gly Gln
 100 105 110

Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 107
 <211> 123
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic antibody

<400> 107

- 28 -

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

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

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

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80

Val Leu Thr Met Thr Asn Met Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

Cys Ala Arg Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
 100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
 115 120

<210> 108
 <211> 123
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic antibody

<400> 108

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

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val

- 29 -

```
<210> 109
<211> 112
<212> PRT
<213> Mus musculus

<400> 109
```

```
<210> 110
<211> 112
<212> PRT
<213> Homo sapiens

<400> 110
```

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

- 30 -

Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser Leu Leu His Ser
20 25 30

Asp Gly Lys Thr Tyr Leu Tyr Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Glu Val Ser Ser Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Gly
85 90 95

Ile His Leu Pro Tyr Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 111
<211> 112
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic antibody

<400> 111

Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly
1 5 10 15

Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
65 70 75 80

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

- 31 -

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 112
 <211> 112
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic antibody

<400> 112

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
 20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

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

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110

<210> 113
 <211> 369
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic nucleic acid molecule

<400> 113

cagggtgaccc tgaaggagtc tggccctgcc ctggtgaagc ccaccagac cctgaccctg 60
 acctgcacct tctctggctt cagcctgagc acctctggca tgggogtggg ctggatccgg 120
 cagccccctg gcaaggccct ggagtggctg gccacatct ggtgggacga cgacaagtcc 180
 tacaacccca gcctgaagag ccggctgacc atcagcaagg acaccagcaa gaaccaggtg 240

- 32 -

gtgtgacca tgaccaacat ggacctgtg gacacagcca cctactactg tgcccggcgg 300
 cagctggggc tcgggagcat tgatgcatg gactactggg gccagggcac cacagtgaca 360
 gtgtccagc 369

<210> 114
 <211> 369
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic nucleic acid molecule

<400> 114
 caggtgaccc tgaaggagtc tggccctgcc ctggtgaagc ccaccagac cctgaccctg 60
 acctgcaccc tgtctggctt cagcctgagc acctctggca tgggcgtggg ctggatccgg 120
 cagccccctg gcaaggccct ggagtggctg gccacatct ggtgggacga cgacaagtcc 180
 tacaaccca gcctgaagag ccggtgacc atcagcaagg acaccagcaa gaaccaggtg 240
 gtgtgacca tgaccaacat ggacctgtg gacacagcca cctactactg tgcccggcgg 300
 cagctggggc tcgggagcat tgatgcatg gactactggg gccagggcac cacagtgaca 360
 gtgtccagc 369

<210> 115
 <211> 336
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic nucleic acid molecule

<400> 115
 gatgtggtga tgaccagag cccctgtcc ctgcctgtga cccctggcga gcctgccagc 60
 atctcctgcc ggagctcca gagcatcctg cactccaatg gcaacaccta cctggagtgg 120
 tacctgcaga agcctggcca gagccccag ctgctgatct acaagggtgc caaccggttc 180
 tccggcgtgc ctgaccggtt cagcggctcc ggcagcggca cagacttcac cctgaagatc 240
 agccgggttg aggtgagga tgtggcgctc tactactgct tocagggcag cctggtgccc 300
 ctgacctttg gccagggcac caagctggag atcaag 336

<210> 116
 <211> 482
 <212> PRT
 <213> Artificial Sequence

- 33 -

<220>

<223> Synthetic polypeptide

<400> 116

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
 1 5 10 15

Thr Val Ala Gln Ala Ala Leu Glu Gln Val Thr Leu Lys Glu Ser Gly
 20 25 30

Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Phe
 35 40 45

Ser Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly Trp Ile Arg
 50 55 60

Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala His Ile Trp Trp Asp
 65 70 75 80

Asp Asp Lys Ser Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser
 85 90 95

Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp
 100 105 110

Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Arg Gln Leu Gly Leu
 115 120 125

Arg Ser Ile Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr
 130 135 140

Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro
 145 150 155 160

Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val
 165 170 175

Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala
 180 185 190

Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly
 195 200 205

Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly
 210 215 220

- 34 -

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys
 225 230 235 240

Val Asp Lys Arg Val Glu Pro Lys Ser Cys Thr Ser Gly His His His
 245 250 255

His His His Gly Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly
 260 265 270

Gly Pro Phe Val Cys Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln
 275 280 285

Pro Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly
 290 295 300

Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser
 305 310 315 320

Glu Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr
 325 330 335

Glu Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp
 340 345 350

Glu Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala
 355 360 365

Thr Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly
 370 375 380

Leu Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser
 385 390 395 400

Gln Met Ala Gln Val Gly Asp Gly Asp Asn Ser Pro Leu Met Asn Asn
 405 410 415

Phe Arg Gln Tyr Leu Pro Ser Leu Pro Gln Ser Val Glu Cys Arg Pro
 420 425 430

Tyr Val Phe Gly Ala Gly Lys Pro Tyr Glu Phe Ser Ile Asp Cys Asp
 435 440 445

Lys Ile Asn Leu Phe Arg Gly Val Phe Ala Phe Leu Leu Tyr Val Ala

PCT/US06/40508

- 35 -

450

455

460

Thr Phe Met Tyr Val Phe Ser Thr Phe Ala Asn Ile Leu Arg Asn Lys
 465 470 475 480

Glu Ser

<210> 117

<211> 481

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 117

Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala
 1 5 10 15

Thr Val Ala Gln Ala Ala Leu Glu Gln Val Thr Leu Lys Glu Ser Gly
 20 25 30

Pro Ala Leu Val Lys Pro Thr Gln Thr Leu Thr Leu Thr Cys Thr Leu
 35 40 45

Ser Gly Phe Ser Leu Ser Thr Ser Gly Met Gly Val Gly Trp Ile Arg
 50 55 60

Gln Pro Pro Gly Lys Ala Leu Glu Trp Leu Ala His Ile Trp Trp Asp
 65 70 75 80

Asp Asp Lys Ser Tyr Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser
 85 90 95

Lys Asp Thr Ser Lys Asn Gln Val Val Leu Thr Met Thr Asn Met Asp
 100 105 110

Pro Val Asp Thr Ala Thr Tyr Tyr Cys Ala Arg Arg Gln Leu Gly Leu
 115 120 125

Arg Ser Ile Asp Ala Met Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr
 130 135 140

Val Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser
 145 150 155 160

- 36 -

Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys
 165 170 175

Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu
 180 185 190

Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu
 195 200 205

Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr
 210 215 220

Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val
 225 230 235 240

Asp Lys Arg Val Glu Pro Lys Ser Cys Thr Ser Gly His His His His
 245 250 255

His His Gly Gly Glu Gln Lys Leu Ile Ser Glu Glu Asp Leu Gly Gly
 260 265 270

Pro Phe Val Cys Glu Tyr Gln Gly Gln Ser Ser Asp Leu Pro Gln Pro
 275 280 285

Pro Val Asn Ala Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
 290 295 300

Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu
 305 310 315 320

Gly Gly Gly Ser Gly Gly Gly Ser Gly Ser Gly Asp Phe Asp Tyr Glu
 325 330 335

Lys Met Ala Asn Ala Asn Lys Gly Ala Met Thr Glu Asn Ala Asp Glu
 340 345 350

Asn Ala Leu Gln Ser Asp Ala Lys Gly Lys Leu Asp Ser Val Ala Thr
 355 360 365

Asp Tyr Gly Ala Ala Ile Asp Gly Phe Ile Gly Asp Val Ser Gly Leu
 370 375 380

Ala Asn Gly Asn Gly Ala Thr Gly Asp Phe Ala Gly Ser Asn Ser Gln

385					390						395					400
Met	Ala	Gln	Val	Gly	Asp	Gly	Asp	Asn	Ser	Pro	Leu	Met	Asn	Asn	Phe	
				405					410					415		
Arg	Gln	Tyr	Leu	Pro	Ser	Leu	Pro	Gln	Ser	Val	Glu	Cys	Arg	Pro	Tyr	
			420					425					430			
Val	Phe	Gly	Ala	Gly	Lys	Pro	Tyr	Glu	Phe	Ser	Ile	Asp	Cys	Asp	Lys	
		435					440					445				
Ile	Asn	Leu	Phe	Arg	Gly	Val	Phe	Ala	Phe	Leu	Leu	Tyr	Val	Ala	Thr	
	450					455						460				
Phe	Met	Tyr	Val	Phe	Ser	Thr	Phe	Ala	Asn	Ile	Leu	Arg	Asn	Lys	Glu	
465					470					475					480	

<210>	118
<211>	243
<212>	PRT
<213>	Artificial Sequence
<220>	
<223>	Synthetic polypeptide
<400>	118

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

- 38 -

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

Gly Val Tyr Tyr Cys Phe Gln Gly Ser Leu Val Pro Leu Thr Phe Gly
 115 120 125

Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val
 130 135 140

Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser
 145 150 155 160

Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln
 165 170 175

Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val
 180 185 190

Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu
 195 200 205

Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu
 210 215 220

Val Thr His Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg
 225 230 235 240

Gly Glu Cys

<210> 119

<211> 5458

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic nucleic acid molecule

<400> 119

gcccaatacg caaacgcct ctcccgcgc gttggccgat tcattaatgc agctggcacg 60

acaggtttcc cgactggaaa gcgggcagtg agcgcaacgc aattaatgtg agttagctca 120

ctcattaggc accccaggct ttacacttta tgcttcggc tcgtatgttg tgtggaattg 180

tgagcggata acaatttcac acaagatcta gctattccag agattacgca aattctat 240

- 39 -

caaggagaca gtcataatga aatacctgct gccgactgca gctgctggtc tgctgctgct	300
ggcggcccag cgggctatgg cttctagaga tgtggtgatg acccagagcc cctgtccct	360
gcctgtgacc cctggcgagc ctgccagcat ctccctgccg agctcccaga gcctcctgca	420
ctccaatggc aacacctacc tggagtggta cctgcagaag cctggccaga gccccagct	480
gctgatctac aagggtgtcca accggttctc cggcgtgcct gaccggttca ggggctccgg	540
cagcggcaca gacttcaccc tgaagatcag ccgggtggag gctgaggatg tgggcgtcta	600
ctactgcttc cagggcagcc tgggtcccct gacctttggc cagggcacca agctggagat	660
caagcgtacg gtggctgcac catctgtctt catcttcccg ccctctgatg agcagttgaa	720
atctggaact gcctctgttg tgtgcctgct gaataacttc tatccagag aggccaaagt	780
acagtggaag gtggataacg ccctccaatc gggtaactcc caggagagtg tcacagagca	840
ggacagcaag gacagcacct acagcctcag cagcacctg acgctgagca aagcagacta	900
cgagaaacac aaagtctacg cctgcgaagt caccatcag ggctgagct cggccgtcac	960
aaagagcttc aacaggggag agtggttaaca attgctagaa ttgtgagcgg ataacaattt	1020
cagcaggtcg agttcttgat aacgaggcgt aaaaaatgaa aaagacagct atcgcgattg	1080
cagtggcact ggctggtttc gctaccgtgg ccagggcggc cctcgagcag gtgaccctga	1140
aggagtctgg cctgccctg gtgaagccca ccagaccct gacctgacc tgcaccttct	1200
ctggcttcag cctgagcacc tctggcatgg gcgtgggctg gatccggcag cccctggca	1260
aggccctgga gtggctggcc cacatctggc gggacgacga caagtctac aacccagcc	1320
tgaagagccg gctgaccatc agcaaggaca ccagcaagaa ccagggtgtg ctgaccatga	1380
ccaacatgga ccctgtggac acagccacct actactgtgc ccggcggcag ctgggcctgc	1440
ggagcattga tgccatggac tactggggcc agggcaccac agtgacagtg tccagcgct	1500
ccaccaaggg cccatcggtc ttccccctgg caacctctc caagagcacc tctgggggca	1560
caggggccct gggctgcctg gtcaaggact acttccccga accggtgacg gtgtcgtgga	1620
actcaggcgc cctgaccagc ggctgcaca ccttccggc tgtcctacag tctcaggac	1680
tctactccct cagcagcgtg gtgaccgtgc cctccagcag cttgggcacc cagacctaca	1740
tctgcaacgt gaatcacaag ccagcaaca ccaaggtgga caagagagtt gagcccaa	1800
cttgtactag tggccaccac caccatcacc atggcgtgga aaaaaactc atctcagaag	1860
aggatctggg tggtttagcca ttcgtttgtg aatatcaagg ccaatcgtct gacctgcctc	1920
aacctcctgt caatgctggc ggggctctg gtgggtggtc tgggtggcggc tctgaggtg	1980
gcggtctga ggggtggcgt tctgaggtg gcggctctga ggggtggcgt tccggtggcg	2040

- 40 -

gctccggttc cgggtgatttt gattatgaaa aaatggcaaa cgctaataag ggggctatga 2100
ccgaaaatgc cgatgaaaac gcgctacagt ctgacgctaa aggcaaactt gattctgtcg 2160
ctactgatta cgggtgctgct atcgatgggt tcattgggtga cgtttccggc cttgctaattg 2220
gtaatggtgc tactggtgat tttgctggct ctaattccca aatggctcaa gtcgggtgacg 2280
gtgataattc acctttaatg aataatttcc gtcaatatat accttctttg cctcagtcgg 2340
ttgaatgtcg cccttatgtc tttggcgctg gtaaaccata tgaattttct attgattgtg 2400
acaaaataaa cttattccgt ggtgtctttg cgtttctttt atatgttgcc acctttatgt 2460
atgtattttc gacgtttgct aacatactgc gtaataagga gtottaagct agctggcgt 2520
cgttttacaa cgtcgtgact gggaaaaccc tggcgttacc caacttaatc gccttgacgc 2580
acatccccct ttgcgcagct ggcgtaatag cgaagaggcc cgcaccgatc gcccttccca 2640
acagttgcgc agcctgaatg gcgaatggcg attccgttgc aatggctggc ggtaatatgt 2700
ttctggatat taccagcaag gccgatagtt tgagttcttc tactcaggca agtgatgtta 2760
ttactaatca aagaagtatt gcgacaacgg ttaatttgcg tgatggacag actcttttac 2820
tcggtggcct cactgattat aaaaacactt ctcaggatc tggcgtagcg ttctgtctta 2880
aaatcccttt aatoggcctc ctgttttagct cccgctctga ttotaacgag gaaagcacgt 2940
tatacgtgct cgtaaaagca accatagtag gcgccctgta gggcgccatt aagcgcgcg 3000
ggtgtggtgg ttaogcgcag cgtgaccgct acacttgcca gcgccctagc gccgcctcct 3060
ttcgctttct tcccttctct tctgcgcacg ttgcgcggct tccccgtca agctctaaat 3120
cgggggctcc ctttaggggt ccgatttagt gctttacggc acctcgacct caaaaaactt 3180
gattaggggt atggttcacg tagtgggcca tcgccctgat agacggtttt tcgccctttg 3240
acgttggagt ccacgttctt taatagtggg ctcttgttcc aaactggaac aacactcaac 3300
cctatctcgg tctattcttt tgatttataa gggattttgc cgatttcggc ctattggtta 3360
aaaaatgagc tgatttaaca aaaatttaac gcgaatttta acaaaatatt aacgtttaca 3420
atttaaatat ttgcttatac aatcttctct tttttggggc ttttctgatt atcaaccggg 3480
gtacatatga ttgacatgct agttttacga ttaccgttca tcgcagggtg cacttttcgg 3540
ggaaatgtgc gcggaacccc tatttgttta tttttctaaa tacattcaaa tatgtatcgg 3600
ctcatgagac aataaccctg ataaatgctt caataatatt gaaaaaggaa gagtatgagt 3660
attcaacatt tcogtgtcgc ccttattccc ttttttgccg catthttgct toctgttttt 3720
gctcaccag aaacgctggg gaaagtaaaa gatgctgaag atcagttggg tgcacgagtg 3780

- 41 -

ggttacatcg aactggatct caacagcggg aagatccttg agagttttcg cccgaagaa 3840
cgttttccaa tgatgagcac ttttaaagtt ctgctatgtg gcgcggtatt atcccgatt 3900
gacgcggggc aagagcaact cggtcgccgc atacactatt ctccagaatga cttgggtgag 3960
tactcaccag tcacagaaaa gcatcttacg gatggcatga cagtaagaga attatgcagt 4020
gctgccataa ccatgagtga taacactgcg gccaaacttac ttctgacaac gatcggagga 4080
ccgaaggagc taaccgcttt ttgacacaac atgggggatc atgtaactcg ccttgatcgt 4140
tggaaccgg agctgaatga agccatacca aacgacgagc gtgacaccac gatgcctgta 4200
gcaatggcaa caacgttgcg caaactatta actggcgaac tacttactct agcttcccg 4260
caacaattaa tagactggat ggaggcggat aaagttgcag gaccacttct gcgctcgcc 4320
cttcggctg gctggtttat tctgataaa tctggagccg gtgagcgtgg gtctcgcggt 4380
atcattgcag cactggggcc agatggtaag cctcccgta tctagttat ctacacgagc 4440
gggagtcagg caactatgga tgaacgaaat agacagatcg ctgagatagg tgcctcactg 4500
attaagcatt ggtaactgtc agaccaagtt tactcatata tacttttagat tgatttaaaa 4560
cttcattttt aatttaaaag gatctagggtg aagatccttt ttgataatct catgacaaa 4620
atcccttaac gtgagttttc gttccactga gcgtcagacc cgtagaaaa gatcaaagga 4680
tcttcttgag atcctttttt tctgcgcgta atctgctgct tgcaaaaaa aaaaccaccg 4740
ctaccagcgg tggtttggtt gccggatcaa gagctaccaa ctctttttcc gaaggtaact 4800
ggcttcagca gagcgcagat accaaatact gtccttctag tctagccgta gttaggccac 4860
cacttcaaga actctgtagc accgcctaca tacctcgctc tgctaatacct gttaccagt 4920
gctgctgcca gtggcgataa gtctgtctt accgggttg actcaagacg atagttaccg 4980
gataaggcgc agcggtcggg ctgaacgggg gggtcgtgca cacagcccag cttggagcga 5040
acgacctaca ccgaactgag atacctacag cgtgagctat gagaaagcgc caccgttccc 5100
gaagggagaa aggcggacag gtatccggtg agcggcaggg tcggaacagg agagcgcagc 5160
agggagcttc cagggggaaa cgctggtat ctttatagtc ctgtcgggtt tcgccacctc 5220
tgacttgagc gtogattttt gtgatgctcg tcaggggggc ggagcctatg gaaaaacgcc 5280
agcaacgcgg cctttttacg gttcctggcc ttttgctggc cttttgctca catgttcttt 5340
cctgcgttat ccctgattc tgtggataac cgtattaccg cctttgagtg agctgatacc 5400
gctcgccgca gccgaacgac cgagcgcagc gagtcaagtga gcgaggaagc ggaagagc 5458

<210> 120

<211> 26

- 42 -

<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<400> 120
ctatggcttc tagagatgtg gtgatg 26

<210> 121
<211> 398
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<400> 121
agctgctggt ctgctgctgc tggcggccca gccggctatg gcttctagag atgtggtgat 60
gaccagagc cccctgtccc tgctgtgac cctggcgag cctgccagca tctcctgccg 120
gagctcccag agcatcctgc actccaatgg caacacctac ctggagtggg acctgcagaa 180
gcctggccag agccccagc tgctgatcta caaggtgtcc aaccggttct ccggcgtgcc 240
tgaccgggttc agcggctccg gcagcggcac agaacttacc ctgaagatca gccgggtgga 300
ggctgaggat gtgggcgtct actactgctt ccagggcagc ctggtgcccc tgacctttgg 360
ccagggcacc aagctggaga tcaagcgtac ggtggctg 398

<210> 122
<211> 398
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<220>
<221> misc_feature
<222> (329)..(330)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (332)..(333)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (335)..(336)
<223> n is a, c, g, or t

<220>

- 43 -

<221> misc_feature
<222> (338)..(339)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (341)..(342)
<223> n is a, c, g, or t

<400> 122
agctgctggg ctgctgctgc tggcggccca gccggctatg gcttctagag atgtgggtgat 60
gaccagagc cccctgtccc tgcctgtgac ccctggcgag cctgccagca tctcctgccg 120
gagctcccag agcatcctgc actccaatgg caacacctac ctggagtggg acctgcagaa 180
gcctggccag agccccagc tgctgatcta caagggtgcc aaccggttct ccggcgtgcc 240
tgaccggttc agcggctccg gcagcggcac agacttcacc ctgaagatca gccgggtgga 300
ggctgaggat gtgggcgtct actactgenn knnknknknk nnkgtgcccc tgacctttgg 360
ccagggcacc aagctggaga tcaagcgtac ggtggctg 398

<210> 123
<211> 82
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (57)..(58)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (60)..(61)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (63)..(64)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (66)..(67)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (69)..(70)
<223> n is a, c, g, or t

- 44 -

<400> 123
cagccaccgt acgcttgatc tccagcttgg tgccctggcc aaaggtcagg ggcacmnnmn 60
nmnnmnnmnn gcagtagtag ac 82

<210> 124
<211> 398
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<220>
<221> misc_feature
<222> (341)..(342)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (344)..(345)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (347)..(348)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (350)..(351)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (353)..(354)
<223> n is a, c, g, or t

<400> 124
agctgctggt ctgctgctgc tggcggccca gccggctatg gcttctagag atgtggtgat 60
gaccagagc cccctgtccc tgctgtgac cctggcgag cctgccagca tctcctgccg 120
gagctcccag agcatcctgc actccaatgg caacacctac ctggagtggg acctgcagaa 180
gcctggccag agccccagc tgctgatcta caagggtgtcc aaccggttct ccggcgtgcc 240
tgaccggttc agcggctccg gcagcggcac agacttcacc ctgaagatca gccgggtgga 300
gggtgaggat gtgggcgtct actactgctt ccagggcagc nnknnknnkn nknnktttgg 360
ccagggcacc aagctggaga tcaagcgtac ggtggctg 398

<210> 125
<211> 82
<212> DNA

- 45 -

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<220>

<221> misc_feature

<222> (45)..(46)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (48)..(49)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (51)..(52)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (54)..(55)

<223> n is a, c, g, or t

<220>

<221> misc_feature

<222> (57)..(58)

<223> n is a, c, g, or t

<400> 125

cagccaccgt acgcttgatc tccagcttgg tgccctggcc aaamnnmnm nmmnnmngc 60

tgccctggaa gcagtagtag ac 82

<210> 126

<211> 27

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic oligonucleotide

<400> 126

gtttatctcg agcaggtgac cctgaag 27

<210> 127

<211> 399

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic nucleic acid molecule

<400> 127

ccgtggccca ggcggccctc gagcaggtga ccctgaagga gtctggccct gccctggtga 60

- 46 -

agcccaccca gaccctgacc ctgacctgca cttctctctgg cttcagcctg agcacctctg 120
gcatggggcgt gggctggatc cggcagcccc ctggcaaggc cctggagtgg ctggcccaca 180
tctggtggga cgacgacaag tctacaacc ccagcctgaa gagccggctg accatcagca 240
aggacaccag caagaaccag gtggtgctga ccatgaccaa catggaccct gtggacagtg 300
cccggcgga gctgggcctg cggagcattg atgccatgga ctactggggc cagggcacca 360
cagtgcagt gtccagcgcc tccaccaagg gcccatcgg 399

<210> 128
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<220>
<221> misc_feature
<222> (306)..(307)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (309)..(310)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (312)..(313)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (315)..(316)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (318)..(319)
<223> n is a, c, g, or t

<400> 128
ccgtggccca ggcggccctc gagcaggatga cctgaagga gtctggccct gccctggtga 60
agcccaccca gaccctgacc ctgacctgca cttctctctgg cttcagcctg agcacctctg 120
gcatggggcgt gggctggatc cggcagcccc ctggcaaggc cctggagtgg ctggcccaca 180
tctggtggga cgacgacaag tctacaacc ccagcctgaa gagccggctg accatcagca 240
aggacaccag caagaaccag gtggtgctga ccatgaccaa catggaccct gtggacagtg 300

- 47 -

cccggnnknn knnknnknnk cggagcattg atgccatgga ctactggggc cagggcacca 360
cagtgcacagt gtccagcgcc tccaccaagg gcccatcgg 399

<210> 129
<211> 102
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (81)..(82)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (84)..(85)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (87)..(88)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (90)..(91)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (93)..(94)
<223> n is a, c, g, or t

<400> 129
ccgatgggcc cttggtggag gcgctggaca ctgtcactgt ggtgccctgg cccagtagt 60
ccatggcatc aatgctccgm nnnnnnnnnn nnnnccgggc ac 102

<210> 130
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<220>
<221> misc_feature
<222> (330)..(331)
<223> n is a, c, g, or t

- 48 -

<220>
<221> misc_feature
<222> (333)..(334)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (336)..(337)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (339)..(340)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (342)..(343)
<223> n is a, c, g, or t

<400> 130
ccgtggccca ggcggccctc gagcaggtga ccctgaagga gtctggccct gccctggtga 60
agcccacca gaccctgacc ctgacctgca cttctctctg cttcagcctg agcacctctg 120
gcatgggcgt gggctggatc cggcagcccc ctggcaaggc cctggagtgg ctggcccaca 180
tctggtggga cgacgacaag tcctacaacc ccagcctgaa gagccggctg accatcagca 240
aggacaccag caagaaccag gtggtgctga ccatgaccaa catggaccct gtggacagtg 300
cccggcggca gctgggcctg cggagcattn nknnknnknn knnktggggc cagggaacca 360
cagtgcagt gtccagcgcc tccaccaagg gcccatcgg 399

<210> 131
<211> 91
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic oligonucleotide

<220>
<221> misc_feature
<222> (57)..(58)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (60)..(61)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (63)..(64)
<223> n is a, c, g, or t

- 49 -

<220>
<221> misc_feature
<222> (66)..(67)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (69)..(70)
<223> n is a, c, g, or t

<400> 131
ccgatgggcc cttggtggag gcgctggaca ctgtcactgt ggtgccctgg ccccamnnnn 60
nnnnnnnnnn aatgctccgc aggccagct g 91

<210> 132
<211> 399
<212> DNA
<213> Artificial Sequence

<220>
<223> Synthetic nucleic acid molecule

<220>
<221> misc_feature
<222> (318)..(319)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (321)..(322)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (324)..(325)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (327)..(328)
<223> n is a, c, g, or t

<220>
<221> misc_feature
<222> (330)..(331)
<223> n is a, c, g, or t

<400> 132
ccgtggccca ggaggccctc gagcaggtga cctgaagga gtctggccct gccctggtga 60
agccaccaca gaccctgacc ctgacctgca ccttctctgg cttcagcctg agcacctctg 120
gcatgggctg gggctggatc cggcagcccc ctggcaaggc cctggagtgg ctggccca 180
tctggtggga cgacgacaag tcctacaacc ccagcctgaa gagccggctg accatcagca 240

- 50 -

aggacaccag caagaaccag gtggtgctga ccatgaccaa catggaccct gtggacagtg 300
 cccggcggca gctgggcnnk nnknnknnkn nkgccatgga ctactggggc cagggcacca 360
 cagtgcagct gtccagcgcc tccaccaagg gcccatcgg 399

<210> 133
 <211> 91
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic oligonucleotide

<220>
 <221> misc_feature
 <222> (69)..(70)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (72)..(73)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (75)..(76)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (78)..(79)
 <223> n is a, c, g, or t

<220>
 <221> misc_feature
 <222> (81)..(82)
 <223> n is a, c, g, or t

<400> 133
 ccgatgggccc cttggtggag gcgctggaca ctgtcactgt ggtgccctgg cccagtagt 60
 ccatggcmnn mnnmnnmnnm nngcccagct g 91

<210> 134
 <211> 330
 <212> PRT
 <213> Homo sapiens

<400> 134

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys
 1 5 10 15

PCT/US2006/040508

- 51 -

Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

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

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 65 70 75 80

Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

Lys Ala Glu Pro Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys
 100 105 110

Pro Ala Pro Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro
 115 120 125

Lys Pro Lys Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys
 130 135 140

Val Val Val Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp
 145 150 155 160

Tyr Val Asp Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu
 165 170 175

Glu Gln Tyr Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu
 180 185 190

His Gln Asp Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn
 195 200 205

Lys Ala Leu Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly
 210 215 220

Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu
 225 230 235 240

Leu Thr Lys Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr
 245 250 255

- 52 -

Pro Ser Asp Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn
 260 265 270

Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe
 275 280 285

Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn
 290 295 300

Val Phe Ser Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr
 305 310 315 320

Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys
 325 330

<210> 135
 <211> 326
 <212> PRT
 <213> Homo sapiens

<400> 135

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

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

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
 65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
 100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 115 120 125

- 53 -

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 130 135 140

Val Ser His Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
 145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
 165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Val His Gln Asp Trp
 180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
 195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
 210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
 225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
 275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
 305 310 315 320

Ser Leu Ser Pro Gly Lys
 325

<210> 136

<211> 327

<212> PRT

<213> Homo sapiens

<400> 136

- 54 -

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1 5 10 15
 Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30
 Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45
 Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser
 50 55 60
 Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
 65 70 75 80
 Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95
 Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Ser Cys Pro Ala Pro
 100 105 110
 Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
 115 120 125
 Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
 130 135 140
 Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
 145 150 155 160
 Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe
 165 170 175
 Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
 180 185 190
 Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
 195 200 205
 Pro Ser Ser Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
 210 215 220
 Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
 225 230 235 240

- 55 -

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
 245 250 255

Ile Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys
 260 265 270

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
 275 280 285

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
 290 295 300

Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
 305 310 315 320

Leu Ser Leu Ser Leu Gly Lys
 325

<210> 137

<211> 326

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 137

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
 1 5 10 15

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
 20 25 30

Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser
 35 40 45

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

Leu Ser Ser Val Val Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr
 65 70 75 80

Tyr Thr Cys Asn Val Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys
 85 90 95

- 56 -

Thr Val Glu Arg Lys Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro
 100 105 110

Pro Val Ala Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
 115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
 130 135 140

Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly
 145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn
 165 170 175

Ser Thr Phe Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
 180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro
 195 200 205

Ser Ser Ile Glu Lys Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu
 210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
 225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
 245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
 260 265 270

Thr Pro Pro Met Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
 275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
 290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
 305 310 315 320

Ser Leu Ser Pro Gly Lys
 325

- 57 -

<210> 138

<211> 449

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 138

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

Thr Leu Thr Leu Thr Cys Thr Leu Ser Gly Phe Ser Leu Ser Thr Ser
 20 25 30

Gly Met Gly Val Gly Trp Phe Arg Gln Pro Pro Gly Lys Gly Leu Glu
 35 40 45

Trp Leu Ala His Ile Trp Trp Asp Asp Asp Lys Ser Tyr Asn Pro Ser
 50 55 60

Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Lys Asn Gln Val
 65 70 75 80

Val Leu Thr Ile Thr Asn Val Asp Pro Val Asp Thr Ala Thr Tyr Tyr
 85 90 95

Cys Ala Arg Arg Gln Leu Gly Thr Arg Gly Thr Asp Ala Met Asp Tyr
 100 105 110

Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 115 120 125

Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg Ser Thr Ser Glu Ser
 130 135 140

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 145 150 155 160

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
 165 170 175

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
 180 185 190

- 58 -

Thr Val Thr Ser Ser Asn Phe Gly Thr Gln Thr Tyr Thr Cys Asn Val
 195 200 205

Asp His Lys Pro Ser Asn Thr Lys Val Asp Lys Thr Val Glu Arg Lys
 210 215 220

Cys Cys Val Glu Cys Pro Pro Cys Pro Ala Pro Pro Val Ala Gly Pro
 225 230 235 240

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
 245 250 255

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser Gln Glu Asp
 260 265 270

Pro Glu Val Gln Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
 275 280 285

Ala Lys Thr Lys Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Val
 290 295 300

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
 305 310 315 320

Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu Pro Ser Ser Ile Glu Lys
 325 330 335

Thr Ile Ser Lys Thr Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
 340 345 350

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
 355 360 365

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
 370 375 380

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Met Leu
 385 390 395 400

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
 405 410 415

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
 420 425 430

- 59 -

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
 435 440 445

Lys

<210> 139

<211> 1407

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthetic nucleic acid molecule

<400> 139

```

atggaatgga gctgggtctt tctcttcttc ctgtcagtaa ctacaggtgt ccactcgag      60
gtgaccctga aggagtctgg ccctggcctg ctgaagccca ccagaccct gaccctgacc      120
tgcaccctgt ctggcttcag cctgagcacc totggcatgg gcgtgggctg gttccggcag      180
ccccctggca agggcctgga gtggctggcc cacatctggt gggacgacga caagtcctac      240
aaccacagcc tgaagagccg gctgaccatc agcaaggaca ccagcaagaa ccaggtggtg      300
ctgaccatca ccaacgtgga ccctgtggac acagccacct actactgtgc ccggcggcag      360
ctgggcacta gggggacgga tgccatggac tactggggcc agggcaccac agtgacagtg      420
tccagcgcac ccaaccaagg cccatccgtc ttccccctgg cgccctgctc caggagcacc      480
tccgagagca cagccgccct gggctgcctg gtcaaggact acttccccga accggtgacg      540
gtgtcgtgga actcaggcgc cctgaccagc ggctgcaca ccttcccggc tgtcctacag      600
tcctcaggac totactccct cagcagcgtg gtgaccgtga cctccagcaa ctttggcacg      660
cagacctaca cctgcaacgt agatcacaag ccagcaaca ccaaggtgga caagacagtt      720
gagcggaaat gctgcgtgga gtgccacca tgcccagcac ctccagtggc cggaccatca      780
gtcttcctgt tcccccaaaa acccaaggac actctcatga totccggac ccctgaggtc      840
acgtgcgtgg tgggtggacgt gagccaggaa gaccccgagg tccagttcaa ctggtacgtg      900
gatggcgtgg aggtgcataa tgccaagaca aagccgcggg aggagcagtt caacagcacg      960
ttcgtgtggt tcagcgtcct cacogtcctg caccaggact ggctgaacgg caaggagtac     1020
aagtgcaggg totccaacaa aggcctcccg tcctccatcg agaaaaccat ctccaaaacc     1080
aaagggcagc ccgagagacc acaggtgtac accctgcccc catcccgga ggagatgacc     1140
aagaaccagg tcagcctgac ctgcctggtc aaaggcttct accccagcga catcgccgtg     1200

```

gagtgggaga gcaatgggca gccggagaac aactacaaga ccacgcctcc catgctggac 1260
 tccgacggct ccttcttctt ctacagcaag ctaaccgtgg acaagagcag gtggcagcag 1320
 gggaatgtct tctcatgctc cgtgatgcat gaggctctgc acaaccacta cacacagaag 1380
 agcctctccc tgtctcctgg taaatga 1407

<210> 140

<211> 219

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthetic polypeptide

<400> 140

Asp Val Val Met Thr Gln Thr Pro Leu Ser Leu Pro Val Thr Pro Gly
 1 5 10 15

Gln Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Ile Leu His Ser
 20 25 30

Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro Gly Gln Ser
 35 40 45

Pro Gln Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser Gly Val Pro
 50 55 60

Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile
 65 70 75 80

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

Thr Arg Val Pro Leu Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys
 100 105 110

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
 115 120 125

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

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
 145 150 155 160

- 61 -

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
 165 170 175

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 180 185 190

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
 195 200 205

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 210 215

<210> 141
 <211> 720
 <212> DNA
 <213> Artificial Sequence

<220>
 <223> Synthetic oligonucleotide

<400> 141
 atgagtgtgc ccactcaggt cctgggggttg ctgctgctgt ggcttacaga tgccagatgc 60
 gatgtggtga tgaccagac cccctgtcc ctgcctgtga cccctggcca gcctgccagc 120
 atctcctgcc ggagctccca gagcatcctg cactccaatg gcaacaccta cctggagtgg 180
 tacctgcaga agcctggcca gagcccccag ctgctgatct acaaggtgtc caaccggttc 240
 tccggcgtgc ctgaccggtt cagcggctcc ggcagcggca cagacttcac cctgaagatc 300
 agccgggttg aggtgagga tgtgggcgtc tactactgcc ttcagactac tegtgtgccc 360
 ctgacctttg gccagggcac caagctggag atcaagcgta cggcggctgc accatctgtc 420
 ttcatcttcc cgccatctga tgagcagttg aaatctggaa ctgcctctgt tgtgtgcctg 480
 ctgaataact tctatcccag agaggccaaa gtacagtgga aggtggataa cgccctccaa 540
 tcgggtaact ccaggagag tgtcacagag caggacagca aggacagcac ctacagcctc 600
 agcagcacc tgacgtgag caaagcagac tacgagaaac acaaagtcta cgctgcgaa 660
 gtcacccatc agggcctgag ctgcgccgtc acaaagagct tcaacagggg agagtgttag 720

<210> 142
 <211> 13
 <212> PRT
 <213> Artificial Sequence

<220>
 <223> Synthetic peptide

<400> 142

Arg Gln Leu Gly Lys Leu Ala Leu Asp Ala Met Asp Tyr
1 5 10

<210> 143
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 143

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 144
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 144

Arg Gln Leu Gly Gln Arg Gln Thr Asp Ala Met Asp Tyr
1 5 10

<210> 145
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 145

Arg Ala Ile Gln Pro Arg Ser Ile Asp Ala Met Asp Tyr
1 5 10

<210> 146
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 146

Arg Gln Leu Gly Leu Arg Ser Ile Asp Ala His Thr Arg
1 5 10

<210> 147
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 147

Arg Gln Leu Gly Gln Pro Ser Val Asp Ala Met Asp Tyr
1 5 10

<210> 148
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 148

Arg Gln Leu Gly Phe Gln Ser Thr Asp Ala Met Asp Tyr
1 5 10

<210> 149
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 149

Arg Gln Leu Gly Gln Ala Gly His Asp Ala Met Asp Tyr
1 5 10

<210> 150
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 150

Arg Gln Leu Gly Asp Asn Val Ala Asp Ala Met Asp Tyr
1 5 10

<210> 151

- 64 -

<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 151

Arg Gln Leu Gly Met Ala Thr Pro Asp Ala Met Asp Tyr
1 5 10

<210> 152
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 152

Arg Gln Leu Gly Ala His Trp Leu Asp Ala Met Asp Tyr
1 5 10

<210> 153
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Synthetic peptide

<400> 153

Arg Gln Leu Gly Pro Glu Pro Gln Asp Ala Met Asp Tyr
1 5 10