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(54) **Title:** HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE

(57) **Abstract:** The invention provides improved agents and methods for treatment of diseases associated with amyloid deposits of Aβ in the brain of a patient. Preferred agents include humanized antibodies.

# HUMANIZED ANTIBODIES THAT RECOGNIZE BETA AMYLOID PEPTIDE

### Related Applications

This application claims the benefit of prior-filed provisional patent

5    application U.S. Serial No. 60/251,892 (filed December 6, 2000) entitled "Humanized Antibodies That Recognize Beta-Amyloid Peptide". The entire content of the above-referenced application is incorporated herein by reference.

### Background of the Invention

10    Alzheimer's disease (AD) is a progressive disease resulting in senile dementia. *See generally* Selkoe, *TINS* 16:403 (1993); Hardy *et al.*, WO 92/13069; Selkoe, *J. Neuropathol. Exp. Neurol.* 53:438 (1994); Duff *et al.*, *Nature* 373:476 (1995); Games *et al.*, *Nature* 373:523 (1995). Broadly speaking, the disease falls into two categories: late onset, which occurs in old age (65 + years) and early onset, which

15    develops well before the senile period, *i.e.*, between 35 and 60 years. In both types of disease, the pathology is the same but the abnormalities tend to be more severe and widespread in cases beginning at an earlier age. The disease is characterized by at least two types of lesions in the brain, neurofibrillary tangles and senile plaques. Neurofibrillary tangles are intracellular deposits of microtubule associated tau protein

20    consisting of two filaments twisted about each other in pairs. Senile plaques (*i.e.*, amyloid plaques) are areas of disorganized neuropil up to 150 μm across with extracellular amyloid deposits at the center which are visible by microscopic analysis of sections of brain tissue. The accumulation of amyloid plaques within the brain is also associated with Down's syndrome and other cognitive disorders.

25    The principal constituent of the plaques is a peptide termed Aβ or β-amyloid peptide. Aβ peptide is a 4-kDa internal fragment of 39-43 amino acids of a larger transmembrane glycoprotein named protein termed amyloid precursor protein (APP). As a result of proteolytic processing of APP by different secretase enzymes, Aβ is primarily found in both a short form, 40 amino acids in length, and a long form,

30    ranging from 42-43 amino acids in length. Part of the hydrophobic transmembrane domain of APP is found at the carboxy end of Aβ, and may account for the ability of Aβ to aggregate into plaques, particularly in the case of the long form. Accumulation of

amyloid plaques in the brain eventually leads to neuronal cell death. The physical symptoms associated with this type of neural deterioration characterize Alzheimer's disease.

5      Several mutations within the APP protein have been correlated with the presence of Alzheimer's disease. See, e.g., Goate et al., Nature 349:704) (1991) (valine[717] to isoleucine); Chartier Harlan et al. Nature 353:844 (1991)) (valine[717] to glycine); Murrell et al., Science 254:97 (1991) (valine[717] to phenylalanine); Mullan et al., Nature Genet. 1:345 (1992) (a double mutation changing lysine[595]-methionine[596] to asparagine[595]-leucine[596]). Such mutations are thought to cause Alzheimer's disease by

10     increased or altered processing of APP to Aβ, particularly processing of APP to increased amounts of the long form of Aβ (i.e., Aβ1-42 and Aβ1-43). Mutations in other genes, such as the presenilin genes, PS1 and PS2, are thought indirectly to affect processing of APP to generate increased amounts of long form Aβ (see Hardy, TINS 20: 154 (1997)).

15     Mouse models have been used successfully to determine the significance of amyloid plaques in Alzheimer's (Games et al., supra, Johnson-Wood et al., Proc. Natl. Acad. Sci. USA 94:1550 (1997)). In particular, when PDAPP transgenic mice, (which express a mutant form of human APP and develop Alzheimer's disease at a young age), are injected with the long form of Aβ, they display both a decrease in the

20     progression of Alzheimer's and an increase in antibody titers to the Aβ peptide (Schenk et al., Nature 400, 173 (1999)). The observations discussed above indicate that Aβ, particularly in its long form, is a causative element in Alzheimer's disease.

McMichael, EP 526,511, proposes administration of homeopathic dosages (less than or equal to $10^{-2}$ mg/day) of Aβ to patients with preestablished AD. In

25     a typical human with about 5 liters of plasma, even the upper limit of this dosage would be expected to generate a concentration of no more than 2 pg/ml. The normal concentration of Aβ in human plasma is typically in the range of 50-200 pg/ml (Seubert et al., Nature 359:325 (1992)). Because EP 526,511's proposed dosage would barely alter the level of endogenous circulating Aβ and because EP 526,511 does not

30     recommend use of an adjuvant, as an immunostimulant, it seems implausible that any therapeutic benefit would result.

Accordingly, there exists the need for new therapies and reagents for the treatment of Alzheimer's disease, in particular, therapies and reagents capable of effecting a therapeutic benefit at physiologic (*e.g.*, non-toxic) doses.

5    **Summary of the Invention**

The present invention features new immunological reagents, in particular, therapeutic antibody reagents for the prevention and treatment of amyloidogenic disease (*e.g.*, Alzheimer's disease). The invention is based, at least in part, on the identification and characterization of two monoclonal antibodies that specifically bind to Aβ peptide

10   and are effective at reducing plaque burden and/or reducing the neuritic dystrophy associated with amyloidogenic disorders. Structural and functional analysis of these antibodies leads to the design of various humanized antibodies for prophylactic and/or therapeutic use. In particular, the invention features humanization of the variable regions of these antibodies and, accordingly provides for humanized immunoglobulin or

15   antibody chains, intact humanized immunoglobulins or antibodies, and functional immunoglobulin or antibody fragments, in particular, antigen binding fragments, of the featured antibodies.

Polypeptides comprising the complementarity determining regions of the featured monoclonal antibodies are also disclosed, as are polynucleotide reagents,

20   vectors and host suitable for encoding said polypeptides.

Methods of treatment of amyloidogenic diseases or disorders (*e.g.*, Alzheimer's disease) are disclosed, as are pharmaceutical compositions and kits for use in such applications.

Also featured are methods of identifying residues within the featured

25   monoclonal antibodies which are important for proper immunologic function and for identifying residues which are amenable to substitution in the design of humanized antibodies having improved binding affinities and/or reduced immunogenicity, when used as therapeutic reagents.

30   **Brief Description of the Drawings**

*Figure 1* depicts an alignment of the amino acid sequences of the light chain of mouse 3D6, humanized 3D6, Kabat ID 109230 and germline A19 antibodies.

CDR regions are indicated by arrows. Bold italics indicate rare murine residues. Bold indicates packing (VH + VL) residues. Solid fill indicates canonical/CDR interacting residues. Asterisks indicate residues selected for backmutation in humanized 3D6, version 1.

5            *Figure 2* depicts an alignment of the amino acid sequences of the heavy chain of mouse 3D6, humanized 3D6, Kabat ID 045919 and germline VH3-23 antibodies. Annotation is the same as for Figure 1.

           *Figure 3* graphically depicts the Aβ binding properties of 3D6, chimeric 3D6 and 10D5. *Figure 3A* is a graph depicting binding of Aβ to chimeric 3D6

10     (PK1614) as compared to murine 3D6. *Figure 3B* is a graph depicting competition of biotinylated 3D6 versus unlabeled 3D6, PK1614 and 10D5 for binding to Aβ.

           *Figure 4* depicts a homology model of 3D6 VH and VL, showing α-carbon backbone trace. VH is shown in as a stippled line, and VL is shown as a solid line. CDR regions are indicated in ribbon form.

15            *Figure 5* graphically depicts the Aβ binding properties of chimeric 3D6 and humanized 3D6. *Figure 5A* depicts ELISA results measuring the binding of humanized 3D6v1 and chimeric 3D6 to aggregated Aβ. *Figure 5B* depicts ELISA results measuring the binding of humanized 3D6v1 and humanized 3D6v2 to aggregated Aβ.

20            *Figure 6* is a graph quantitating the binding of humanized 3D6 and chimeric 3D6 to Aβ plaques from brain sections of PDAPP mice.

           *Figure 7* is a graph showing results of a competitive binding assay testing the ability of humanized 3D6 versions 1 and 2, chimeric 3D6, murine 3D6, and 10D5 to compete with murine 3D6 for binding to Aβ.

25            *Figure 8* graphically depicts of an *ex vivo* phagocytosis assay testing the ability of humanized 3D6v2, chimeric 3D6, and human IgG to mediate the uptake of Aβ by microglial cells.

           *Figure 9* depicts an alignment of the 10D5 VL and 3D6 VL amino acid sequences. Bold indicates residues that match 10D5 exactly.

30            *Figure 10* depicts an alignment of the 10D5 VH and 3D6 VH amino acid sequences. Bold indicates residues that match 10D5 exactly.

## Detailed Description of the Invention

The present invention features new immunological reagents and methods
for preventing or treating Alzheimer's disease or other amyloidogenic diseases. The
invention is based, at least in part, on the characterization of two monoclonal
immunoglobulins, 3D6 and 10D5, effective at binding beta amyloid protein (Aβ) (e.g.,
binding soluble and/or aggregated Aβ), mediating phagocytosis (e.g., of aggregated Aβ),
reducing plaque burden and/or reducing neuritic dystrophy (e.g., in patient). The
invention is further based on the determination and structural characterization of the
primary and secondary structure of the variable light and heavy chains of these
immunoglobulins and the identification of residues important for activity and
immunogenicity.

Immunoglobulins are featured which include a variable light and/or
variable heavy chain of the preferred monoclonal immunoglobulins described herein.
Preferred immunoglobulins, e.g., therapeutic immunoglobulins, are featured which
include a humanized variable light and/or humanized variable heavy chain. Preferred
variable light and/or variable heavy chains include a complementarity determining
region (CDR) from the monoclonal immunoglobulin (e.g., donor immunoglobulin) and
variable framework regions substantially from a human acceptor immunoglobulin. The
phrase "substantially from a human acceptor immunoglobulin" means that the majority
or key framework residues are from the human acceptor sequence, allowing however,
for substitution of residues at certain positions with residues selected to improve activity
of the humanized immunoglobulin (e.g., alter activity such that it more closely mimics
the activity of the donor immunoglobulin) or selected to decrease the immunogenicity of
the humanized immunoglobulin.

In one embodiment, the invention features a humanized immunoglobulin
light or heavy chain that includes 3D6 variable region complementarity determining
regions (CDRs) (i.e., includes one, two or three CDRs from the light chain variable
region sequence set forth as SEQ ID NO:2 or includes one, two or three CDRs from the
heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable
framework region substantially from a human acceptor immunoglobulin light or heavy

chain sequence, provided that at least one residue of the framework residue is backmutated to a corresponding murine residue, wherein said backmutation does not substantially affect the ability of the chain to direct Aβ binding.

In another embodiment, the invention features a humanized

5    immunoglobulin light or heavy chain that includes 3D6 variable region complementarity determining regions (CDRs) (e.g., includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:2 and/or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a variable framework region substantially from a human acceptor

10    immunoglobulin light or heavy chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 light or heavy chain variable region sequence, where the framework residue is selected from the group consisting of (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue (e.g., identified by modeling

15    the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); and (d) a residue participating in the VL-VH interface.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 3D6 variable region CDRs and variable framework regions from a human acceptor immunoglobulin light or heavy

20    chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 light or heavy chain variable region sequence, where the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region, for example a residue capable of interacting

25    with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, or a glycoslyation site residue on the surface of the structural model.

In another embodiment, the invention features a humanized

30    immunoglobulin light chain that includes 3D6 variable region CDRs (e.g., from the 3D6 light chain variable region sequence set forth as SEQ ID NO:2), and includes a human acceptor immunoglobulin variable framework region, provided that at least one

framework residue selected from the group consisting of L1, L2, L36 and L46 (Kabat numbering convention) is substituted with the corresponding amino acid residue from the mouse 3D6 light chain variable region sequence. In another embodiment, the invention features a humanized immunoglobulin heavy chain that includes 3D6 variable

5    region CDRs (*e.g.*, from the 3D6 heavy chain variable region sequence set forth as SEQ ID NO:4), and includes a human acceptor immunoglobulin variable framework region, provided that at least one framework residue selected from the group consisting of H49, H93 and H94 (Kabat numbering convention) is substituted with the corresponding amino acid residue from the mouse 3D6 heavy chain variable region sequence.

10          Preferred light chains include kappa II framework regions of the subtype kappa II (Kabat convention), for example, framework regions from the acceptor immunoglobulin Kabat ID 019230, Kabat ID 005131, Kabat ID 005058, Kabat ID 005057, Kabat ID 005059, Kabat ID U21040 and Kabat ID U41645. Preferred heavy chains include framework regions of the subtype III (Kabat convention), for example,

15    framework regions from the acceptor immunoglobulin Kabat ID 045919, Kabat ID 000459, Kabat ID 000553, Kabat ID 000386 and Kabat ID M23691.

In one embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 10D5 variable region complementarity determining regions (CDRs) (*i.e.*, includes one, two or three CDRs from the light chain variable

20    region sequence set forth as SEQ ID NO:14 or includes one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:16), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence, provided that at least one residue of the framework residue is backmutated to a corresponding murine residue, wherein said backmutation does not

25    substantially affect the ability of the chain to direct Aβ binding.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 10D5 variable region complementarity determining regions (CDRs) (*e.g.*, includes one, two or three CDRs from the light chain variable region sequence set forth as SEQ ID NO:14 and/or includes

30    one, two or three CDRs from the heavy chain variable region sequence set forth as SEQ ID NO:16), and includes a variable framework region substantially from a human acceptor immunoglobulin light or heavy chain sequence, provided that at least one

framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 light or heavy chain variable region sequence, where the framework residue is selected from the group consisting of (a) a residue that non-covalently binds antigen directly; (b) a residue adjacent to a CDR; (c) a CDR-interacting residue (*e.g.*, identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); and (d) a residue participating in the VL-VH interface.

In another embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 10D5 variable region CDRs and variable framework regions from a human acceptor immunoglobulin light or heavy chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 10D5 light or heavy chain variable region sequence, where the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the variable region, for example a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, or a glycoslyation site residue on the surface of the structural model.

In another embodiment, the invention features, in addition to the substitutions described above, a substitution of at least one rare human framework residue. For example, a rare residue can be substituted with an amino acid residue which is common for human variable chain sequences at that position. Alternatively, a rare residue can be substituted with a corresponding amino acid residue from a homologous germline variable chain sequence (*e.g.*, a rare light chain framework residue can be substituted with a corresponding germline residue from an A1, A17, A18, A2, or A19 germline immunoglobulin sequence or a rare heavy chain framework residue can be substituted with a corresponding germline residue from a VH3-48, VH3-23, VH3-7, VH3-21 or VH3-11 germline immunoglobulin sequence.

In another embodiment, the invention features a humanized immunoglobulin that includes a light chain and a heavy chain, as described above, or an antigen-binding fragment of said immunoglobulin. In an exemplary embodiment, the humanized immunoglobulin binds (*e.g.*, specifically binds) to beta amyloid peptide (Aβ)

with a binding affinity of at least $10^7$ $M^{-1}$, $10^8$ $M^{-1}$, or $10^9$ $M^{-1}$. In another embodiment, the immunoglobulin or antigen binding fragment includes a heavy chain having isotype γ1. In another embodiment, the immunoglobulin or antigen binding fragment binds (e.g., specifically binds) to both soluble beta amyloid peptide (Aβ) and aggregated Aβ. In

5   another embodiment, the immunoglobulin or antigen binding fragment mediates phagocytosis (e.g., induces phagocytosis) of beta amyloid peptide (Aβ). In yet another embodiment, the immunoglobulin or antigen binding fragment crosses the blood-brain barrier in a subject. In yet another embodiment, the immunoglobulin or antigen binding fragment reduces both beta amyloid peptide (Aβ) burden and neuritic dystrophy in a

10  subject.

In another embodiment, the invention features chimeric immunoglobulins that include 3D6 variable regions (e.g., the variable region sequences set forth as SEQ ID NO:2 or SEQ ID NO:4). In yet another embodiment, the invention features an immunoglobulin, or antigen-binding fragment thereof, including a variable heavy chain

15  region as set forth in SEQ ID NO:8 and a variable light chain region as set forth in SEQ ID NO:5. In yet another embodiment, the invention features an immunoglobulin, or antigen-binding fragment thereof, including a variable heavy chain region as set forth in SEQ ID NO:12 and a variable light chain region as set forth in SEQ ID NO:11. In another embodiment, the invention features chimeric immunoglobulins that include

20  10D5 variable regions (e.g., the variable region sequences set forth as SEQ ID NO:14 or SEQ ID NO:16). In yet another embodiment, the immunoglobulin, or antigen-binding fragment thereof, further includes constant regions from IgG1.

The immunoglobulins described herein are particularly suited for use in therapeutic methods aimed at preventing or treating amyloidogenic diseases. In one

25  embodiment, the invention features a method of preventing or treating an amyloidogenic disease (e.g., Alzheimer's disease) that involves administering to the patient an effective dosage of a humanized immunoglobulin as described herein. In another embodiment, the invention features pharmaceutical compositions that include a humanized immunoglobulin as described herein and a pharmaceutical carrier. Also featured are

30  isolated nucleic acid molecules, vectors and host cells for producing the immunoglobulins or immunoglobulin fragments or chains described herein, as well as

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methods for producing said immunoglobulins, immunoglobulin fragments or
immunoglobulin chains

The present invention further features a method for identifying 3D6 or
10D5 residues amenable to substitution when producing a humanized 3D6 or 10D5

5    immunoglobulin, respectively. For example, a method for identifying variable
framework region residues amenable to substitution involves modeling the three-
dimensional structure of the 3D6 or 10D5 variable region on a solved homologous
immunoglobulin structure and analyzing said model for residues capable of affecting
3D6 or 10D5 immunoglobulin variable region conformation or function, such that

10   residues amenable to substitution are identified. The invention further features use of
the variable region sequence set forth as SEQ ID NO:2 or SEQ ID NO:4, or any portion
thereof, in producing a three-dimensional image of a 3D6 immunoglobulin, 3D6
immunoglobulin chain, or domain thereof. Also featured is the use of the variable
region sequence set forth as SEQ ID NO:14 or SEQ ID NO:16, or any portion thereof,

15   in producing a three-dimensional image of a 10D5 immunoglobulin, 10D5
immunoglobulin chain, or domain thereof.


Prior to describing the invention, it may be helpful to an understanding
thereof to set forth definitions of certain terms to be used hereinafter.

20           The term "immunoglobulin" or "antibody" (used interchangeably herein)
refers to an antigen-binding protein having a basic four-polypeptide chain structure
consisting of two heavy and two light chains, said chains being stabilized, for example,
by interchain disulfide bonds, which has the ability to specifically bind antigen. Both
heavy and light chains are folded into domains. The term "domain" refers to a globular

25   region of a heavy or light chain polypeptide comprising peptide loops (e.g., comprising
3 to 4 peptide loops) stabilized, for example, by β-pleated sheet and/or intrachain
disulfide bond. Domains are further referred to herein as "constant" or "variable", based
on the relative lack of sequence variation within the domains of various class members
in the case of a "constant" domain, or the significant variation within the domains of

30   various class members in the case of a "variable" domain. "Constant" domains on the
light chain are referred to interchangeably as "light chain constant regions", "light chain
constant domains", "CL" regions or "CL" domains). "Constant" domains on the heavy

chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "CH" regions or "CH" domains). "Variable" domains on the light chain are referred to interchangeably as "light chain variable regions", "light chain variable domains", "VL" regions or "VL" domains). "Variable" domains on the heavy chain are referred to interchangeably as "heavy chain constant regions", "heavy chain constant domains", "CH" regions or "CH" domains).

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The term "region" refers to a part or portion of an antibody chain and includes constant or variable domains as defined herein, as well as more discrete parts or portions of said domains. For example, light chain variable domains or regions include "complementarity determining regions" or "CDRs" interspersed among "framework regions" or "FRs", as defined herein.

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Immunoglobulins or antibodies can exist in monomeric or polymeric form. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody binds antigen or competes with intact antibody (*i.e.*, with the intact antibody from which they were derived) for antigen binding (*i.e.*, specific binding). The term "conformation" refers to the tertiary structure of a protein or polypeptide (*e.g.*, an antibody, antibody chain, domain or region thereof). For example, the phrase "light (or heavy) chain conformation" refers to the tertiary structure of a light (or heavy) chain variable region, and the phrase "antibody conformation" or "antibody fragment conformation" refers to the tertiary structure of an antibody or fragment thereof.

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"Specific binding" of an antibody mean that the antibody exhibits appreciable affinity for antigen or a preferred epitope and, preferably, does not exhibit significant crossreactivity. "Appreciable" or preferred binding include binding with an affinity of at least $10^6$, $10^7$, $10^8$, $10^9$ M$^{-1}$, or $10^{10}$ M$^{-1}$. Affinities greater than $10^7$ M$^{-1}$, preferably greater than $10^8$ M$^{-1}$ are more preferred. Values intermediate of those set forth herein are also intended to be within the scope of the present invention and a preferred binding affinity can be indicated as a range of affinities, for example, $10^6$ to $10^{10}$ M$^{-1}$, preferably $10^7$ to $10^{10}$ M$^{-1}$, more preferably $10^8$ to $10^{10}$ M$^{-1}$. An antibody that "does not exhibit significant crossreactivity" is one that will not appreciably bind to an undesirable entity (*e.g.*, an undesirable proteinaceous entity). For example, an antibody that specifically binds to Aβ will appreciably bind Aβ but will not significantly react

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with non-Aβ proteins or peptides (*e.g.*, non-Aβ proteins or peptides included in plaques). An antibody specific for a preferred epitope will, for example, not significantly crossreact with remote epitopes on the same protein or peptide. Specific binding can be determined according to any art-recognized means for determining such

5     binding. Preferably, specific binding is determined according to Scatchard analysis and/or competitive binding assays.

Binding fragments are produced by recombinant DNA techniques, or by enzymatic or chemical cleavage of intact immunoglobulins. Binding fragments include Fab, Fab', F(ab')₂, Fabc, Fv, single chains, and single-chain antibodies. Other than

10    "bispecific" or "bifunctional" immunoglobulins or antibodies, an immunoglobulin or antibody is understood to have each of its binding sites identical. A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See,

15    *e.g.*, Songsivilai & Lachmann, *Clin. Exp. Immunol.* 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.* 148, 1547-1553 (1992).

The term "humanized immunoglobulin" or "humanized antibody" refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain (*i.e.*, at least one humanized light or heavy chain). The term

20    "humanized immunoglobulin chain" or "humanized antibody chain" (*i.e.*, a "humanized immunoglobulin light chain" or "humanized immunoglobulin heavy chain") refers to an immunoglobulin or antibody chain (*i.e.*, a light or heavy chain, respectively) having a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity determining regions (CDRs) (*e.g.*, at

25    least one CDR, preferably two CDRs, more preferably three CDRs) substantially from a non-human immunoglobulin or antibody, and further includes constant regions (*e.g.*, at least one constant region or portion thereof, in the case of a light chain, and preferably three constant regions in the case of a heavy chain). The term "humanized variable region" (*e.g.*, "humanized light chain variable region" or "humanized heavy chain

30    variable region") refers to a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and complementarity

determining regions (CDRs) substantially from a non-human immunoglobulin or antibody.

The phrase "substantially from a human immunoglobulin or antibody" or "substantially human" means that, when aligned to a human immunoglobulin or
5    antibody amino sequence for comparison purposes, the region shares at least 80-90%, preferably 90-95%, more preferably 95-99% identity (*i.e.*, local sequence identity) with the human framework or constant region sequence, allowing, for example, for conservative substitutions, consensus sequence substitutions, germline substitutions, backmutations, and the like. The introduction of conservative substitutions, consensus
10   sequence substitutions, germline substitutions, backmutations, and the like, is often referred to as "optimization" of a humanized antibody or chain. The phrase "substantially from a non-human immunoglobulin or antibody" or "substantially non-human" means having an immunoglobulin or antibody sequence at least 80-95%, preferably 90-95%, more preferably, 96%, 97%, 98%, or 99% identical to that of a non-
15   human organism, *e.g.*, a non-human mammal.

Accordingly, all regions or residues of a humanized immunoglobulin or antibody, or of a humanized immunoglobulin or antibody chain, except possibly the CDRs, are substantially identical to the corresponding regions or residues of one or more native human immunoglobulin sequences. The term "corresponding region" or
20   "corresponding residue" refers to a region or residue on a second amino acid or nucleotide sequence which occupies the same (*i.e.*, equivalent) position as a region or residue on a first amino acid or nucleotide sequence, when the first and second sequences are optimally aligned for comparison purposes.

The terms "humanized immunoglobulin" or "humanized antibody" are
25   not intended to encompass chimeric immunoglobulins or antibodies, as defined *infra*. Although humanized immunoglobulins or antibodies are chimeric in their construction (*i.e.*, comprise regions from more than one species of protein), they include additional features (*i.e.*, variable regions comprising donor CDR residues and acceptor framework residues) not found in chimeric immunoglobulins or antibodies, as defined herein.

30   The term "significant identity" means that two polypeptide sequences, when optimally aligned, such as by the programs GAP or BESTFIT using default gap weights, share at least 50-60% sequence identity, preferably 60-70% sequence identity,

more preferably 70-80% sequence identity, more preferably at least 80-90% identity, even more preferably at least 90-95% identity, and even more preferably at least 95% sequence identity or more (*e.g.,* 99% sequence identity or more). The term "substantial identity" means that two polypeptide sequences, when optimally aligned, such as by the

5      programs GAP or BESTFIT using default gap weights, share at least 80-90% sequence identity, preferably 90-95% sequence identity, and more preferably at least 95% sequence identity or more (*e.g.,* 99% sequence identity or more). For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and

10     reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters. The terms "sequence identity" and "sequence identity" are used

15     interchangeably herein.

Optimal alignment of sequences for comparison can be conducted, *e.g.,* by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci.*

20     *USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, WI), or by visual inspection (*see generally* Ausubel *et al.,* Current Protocols in Molecular Biology). One example of algorithm that is suitable for determining percent sequence identity and sequence

25     similarity is the BLAST algorithm, which is described in Altschul *et al., J. Mol. Biol.* 215:403 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (publicly accessible through the National Institutes of Health NCBI internet server). Typically, default program parameters can be used to perform the sequence comparison, although customized

30     parameters can also be used. For amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an expectation (E) of 10, and the BLOSUM62 scoring matrix (*see* Henikoff & Henikoff, *Proc. Natl. Acad. Sci. USA* 89:10915 (1989)).

Preferably, residue positions which are not identical differ by conservative amino acid substitutions. For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic sidechains): leu, met, ala, val, leu, ile; Group II (neutral

5    hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a

10   member of another.

Preferably, humanized immunoglobulins or antibodies bind antigen with an affinity that is within a factor of three, four, or five of that of the corresponding non-human antibody. For example, if the nonhuman antibody has a binding affinity of $10^9$ $M^{-1}$, humanized antibodies will have a binding affinity of at least $3 \times 10^9 M^{-1}$, $4 \times 10^9 M^{-1}$

15   $^1$ or $10^9 M^{-1}$. When describing the binding properties of an immunoglobulin or antibody chain, the chain can be described based on its ability to "direct antigen (e.g., Aβ) binding". A chain is said to "direct antigen binding" when it confers upon an intact immunoglobulin or antibody (or antigen binding fragment thereof) a specific binding property or binding affinity. A mutation (e.g., a backmutation) is said to substantially

20   affect the ability of a heavy or light chain to direct antigen binding if it affects (e.g., decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by at least an order of magnitude compared to that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation. A mutation "does not substantially affect (e.g.,

25   decrease) the ability of a chain to direct antigen binding" if it affects (e.g., decreases) the binding affinity of an intact immunoglobulin or antibody (or antigen binding fragment thereof) comprising said chain by only a factor of two, three, or four of that of the antibody (or antigen binding fragment thereof) comprising an equivalent chain lacking said mutation.

30   The term "chimeric immunoglobulin" or antibody refers to an immunoglobulin or antibody whose light and heavy chains are derived from different species. Chimeric immunoglobulins or antibodies can be constructed, for example by

15

genetic engineering, from immunoglobulin gene segments belonging to different species.

An "antigen" is an entity (e.g., a protenaceous entity or peptide) to which an antibody specifically binds.

5       The term "epitope" or "antigenic determinant" refers to a site on an antigen to which an immunoglobulin or antibody (or antigen binding fragment thereof) specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents

10      whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. See, e.g., Epitope Mapping Protocols in

15      Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996).

Antibodies that recognize the same epitope can be identified in a simple immunoassay showing the ability of one antibody to block the binding of another antibody to a target antigen, i.e., a competitive binding assay. Competitive binding is determined in an assay in which the immunoglobulin under test inhibits specific binding

20      of a reference antibody to a common antigen, such as Aβ. Numerous types of competitive binding assays are known, for example: solid phase direct or indirect radioimmunoassay (RIA), solid phase direct or indirect enzyme immunoassay (EIA), sandwich competition assay (see Stahli et al., Methods in Enzymology 9:242 (1983)); solid phase direct biotin-avidin EIA (see Kirkland et al., J. Immunol. 137:3614 (1986));

25      solid phase direct labeled assay, solid phase direct labeled sandwich assay (see Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Press (1988)); solid phase direct label RIA using I-125 label (see Morel et al., Mol. Immunol. 25(1):7 (1988)); solid phase direct biotin-avidin EIA (Cheung et al., Virology 176:546 (1990)); and direct labeled RIA. (Moldenhauer et al., Scand. J. Immunol. 32:77 (1990)).

30      Typically, such an assay involves the use of purified antigen bound to a solid surface or cells bearing either of these, an unlabeled test immunoglobulin and a labeled reference immunoglobulin. Competitive inhibition is measured by determining the amount of

label bound to the solid surface or cells in the presence of the test immunoglobulin. Usually the test immunoglobulin is present in excess. Usually, when a competing antibody is present in excess, it will inhibit specific binding of a reference antibody to a common antigen by at least 50-55%, 55-60%, 60-65%, 65-70% 70-75% or more.

5        An epitope is also recognized by immunologic cells, for example, B cells and/or T cells. Cellular recognition of an epitope can be determined by *in vitro* assays that measure antigen-dependent proliferation, as determined by $^3$H-thymidine incorporation, by cytokine secretion, by antibody secretion, or by antigen-dependent killing (cytotoxic T lymphocyte assay).

10       Exemplary epitopes or antigenic determinants can be found within the human amyloid precursor protein (APP), but are preferably found within the Aβ peptide of APP. Multiple isoforms of APP exist, for example APP$^{695}$ APP$^{751}$ and APP$^{770}$. Amino acids within APP are assigned numbers according to the sequence of the APP$^{770}$ isoform (see *e.g.*, GenBank Accession No. P05067, also set forth as SEQ ID NO:38).

15  Aβ (also referred to herein as beta amyloid peptide and A-beta) peptide is a ~4-kDa internal fragment of 39-43 amino acids of APP (Aβ39, Aβ40, Aβ41, Aβ42 and Aβ43). Aβ40, for example, consists of residues 672-711 of APP and Aβ42 consists of residues 673-713 of APP. As a result of proteolytic processing of APP by different secretase enzymes *iv vivo* or *in situ*, Aβ is found in both a "short form", 40 amino acids in length,

20  and a "long form", ranging from 42-43 amino acids in length. Preferred epitopes or antigenic determinants, as described herein, are located within the N-terminus of the Aβ peptide and include residues within amino acids 1-10 of Aβ, preferably from residues 1-3, 1-4, 1-5, 1-6, 1-7 or 3-7 of Aβ42. Additional referred epitopes or antigenic determinants include residues 2-4, 5, 6, 7 or 8 of Aβ, residues 3-5, 6, 7, 8 or 9 of Aβ, or

25  residues 4-7, 8, 9 or 10 of Aβ42.

The term "amyloidogenic disease" includes any disease associated with (or caused by) the formation or deposition of insoluble amyloid fibrils. Exemplary amyloidogenic diseases include, but are not limited to systemic amyloidosis, Alzheimer's disease, mature onset diabetes, Parkinson's disease, Huntington's disease,

30  fronto-temporal dementia, and the prion-related transmissible spongiform encephalopathies (kuru and Creutzfeldt-Jacob disease in humans and scrapie and BSE in sheep and cattle, respectively). Different amyloidogenic diseases are defined or

characterized by the nature of the polypeptide component of the fibrils deposited. For
example, in subjects or patients having Alzheimer's disease, β-amyloid protein (*e.g.*,
wild-type, variant, or truncated β-amyloid protein) is the characterizing polypeptide
component of the amyloid deposit. Accordingly, Alzheimer's disease is an example of a

5      "disease characterized by deposits of Aβ" or a "disease associated with deposits of Aβ",
*e.g.*, in the brain of a subject or patient. The terms "β-amyloid protein", "β-amyloid
peptide", "β-amyloid", "Aβ" and "Aβ peptide" are used interchangeably herein.

       The term "effective dose" or "effective dosage" is defined as an amount
sufficient to achieve or at least partially achieve the desired effect. The term

10     "therapeutically effective dose" is defined as an amount sufficient to cure or at least
partially arrest the disease and its complications in a patient already suffering from the
disease. Amounts effective for this use will depend upon the severity of the infection
and the general state of the patient's own immune system.

       The term "patient" includes human and other mammalian subjects that

15     receive either prophylactic or therapeutic treatment.

       "Soluble" or "dissociated" Aβ refers to non-aggregating or disaggregated
Aβ polypeptide. "Insoluble" Aβ refers to aggregating Aβ polypeptide, for example, Aβ
held together by noncovalent bonds. Aβ (*e.g.*, Aβ42) is believed to aggregate, at least in
part, due to the presence of hydrophobic residues at the C-terminus of the peptide (part

20     of the transmembrane domain of APP). One method to prepare soluble Aβ is to dissolve
lyophilized peptide in neat DMSO with sonication. The resulting solution is centrifuged
to remove any insoluble particulates.


       ## I. Immunological and Therapeutic Reagents

25     Immunological and therapeutic reagents of the invention comprise or
consist of immunogens or antibodies, or functional or antigen binding fragments thereof,
as defined herein. The basic antibody structural unit is known to comprise a tetramer of
subunits. Each tetramer is composed of two identical pairs of polypeptide chains, each
pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The

30     amino-terminal portion of each chain includes a variable region of about 100 to 110 or
more amino acids primarily responsible for antigen recognition. The carboxy-terminal

portion of each chain defines a constant region primarily responsible for effector function.

Light chains are classified as either kappa or lambda and are about 230 residues in length. Heavy chains are classified as gamma ($\gamma$), mu ($\mu$), alpha ($\alpha$), delta

5    ($\delta$), or epsilon ($\varepsilon$), are about 450-600 residues in length, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Both heavy and light chains are folded into domains. The term "domain" refers to a globular region of a protein, for example, an immunoglobulin or antibody. Immunoglobulin or antibody domains include, for example, 3 or four peptide loops stabilized by $\beta$-pleated sheet and an

10   interchain disulfide bond. Intact light chains have, for example, two domains ($V_L$ and $C_L$) and intact heavy chains have, for example, four or five domains ($V_H$, $C_H1$, $C_H2$, and $C_H3$).

Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also

15   including a "D" region of about 10 more amino acids. (*See generally, Fundamental Immunology* (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989), Ch. 7, incorporated by reference in its entirety for all purposes).

The variable regions of each light/heavy chain pair form the antibody binding site. Thus, an intact antibody has two binding sites. Except in bifunctional or

20   bispecific antibodies, the two binding sites are the same. The chains all exhibit the same general structure of relatively conserved framework regions (FR) joined by three hypervariable regions, also called complementarity determining regions or CDRs. Naturally-occurring chains or recombinantly produced chains can be expressed with a leader sequence which is removed during cellular processing to produce a mature chain.

25   Mature chains can also be recombinantly produced having a non-naturally occurring leader sequence, for example, to enhance secretion or alter the processing of a particular chain of interest.

The CDRs of the two mature chains of each pair are aligned by the framework regions, enabling binding to a specific epitope. From N-terminal to C-

30   terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. "FR4" also is referred to in the art as the D/J region of the variable heavy chain and the J region of the variable light chain. The assignment of

amino acids to each domain is in accordance with the definitions of Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991). An alternative structural definition has been proposed by Chothia *et al., J. Mol. Biol.* 196:901 (1987); *Nature* 342:878 (1989); and *J. Mol. Biol.* 186:651 (1989)

5   (hereinafter collectively referred to as "Chothia *et al.*" and incorporated by reference in their entirety for all purposes).


### A. Aβ Antibodies

Therapeutic agents of the invention include antibodies that specifically

10   bind to Aβ or other component of amyloid plaques. Such antibodies can be monoclonal or polyclonal. Some such antibodies bind specifically to the aggregated form of Aβ without binding to the soluble form. Some bind specifically to the soluble form without binding to the aggregated form. Some bind to both aggregated and soluble forms. Some such antibodies bind to a naturally occurring short form of Aβ (*i.e.*, Aβ39, 40 or 41)

15   without binding to a naturally occurring long form of Aβ (*i.e.*, Aβ42 and Aβ43). Some antibodies bind to a long form of Aβ without binding to a short form. Some antibodies bind to Aβ without binding to full-length amyloid precursor protein. Antibodies used in therapeutic methods preferably have an intact constant region or at least sufficient of the constant region to interact with an Fc receptor. Human isotype IgG1 is preferred

20   because of it having highest affinity of human isotypes for the FcRI receptor on phagocytic cells. Bispecific Fab fragments can also be used, in which one arm of the antibody has specificity for Aβ, and the other for an Fc receptor. Preferred antibodies bind to Aβ with a binding affinity greater than (or equal to) about $10^6$, $10^7$, $10^8$, $10^9$, or $10^{10}$ $M^{-1}$ (including affinities intermediate of these values).

25                         Polyclonal sera typically contain mixed populations of antibodies binding to several epitopes along the length of Aβ. However, polyclonal sera can be specific to a particular segment of Aβ, such as Aβ1-10. Monoclonal antibodies bind to a specific epitope within Aβ that can be a conformational or nonconformational epitope. Prophylactic and therapeutic efficacy of antibodies can be tested using the transgenic

30   animal model procedures described in the Examples. Preferred monoclonal antibodies bind to an epitope within residues 1-10 of Aβ (with the first N terminal residue of

20

natural Aβ designated 1). Some preferred monoclonal antibodies bind to an epitope
within amino acids 1-5, and some to an epitope within 5-10. Some preferred antibodies
bind to epitopes within amino acids 1-3, 1-4, 1-5, 1-6, 1-7 or 3-7. Some preferred
antibodies bind to an epitope starting at resides 1-3 and ending at residues 7-11 of Aβ.

5       Less preferred antibodies include those binding to epitopes with residues 10-15, 15-20,
25-30, 10-20, 20, 30, or 10-25 of Aβ. It is recommended that such antibodies be
screened for activity in the mouse models described in the Examples before use. For
example, it has been found that certain antibodies to epitopes within residues 10-18, 16-
24, 18-21 and 33-42 lack activity (e.g., lack the ability to reduce plaque burden and/or

10      resolve the neuritic pathology associated with Alzheimer's disease). In some methods,
multiple monoclonal antibodies having binding specificities to different epitopes are
used. Such antibodies can be administered sequentially or simultaneously. Antibodies
to amyloid components other than Aβ can also be used (e.g., administered or co-
administered). For example, antibodies can be directed to the amyloid associated

15      protein synuclein.

        When an antibody is said to bind to an epitope within specified residues,
such as Aβ 1-5 for example, what is meant is that the antibody specifically binds to a
polypeptide containing the specified residues (i.e., Aβ 1-5 in this an example). Such an
antibody does not necessarily contact every residue within Aβ 1-5. Nor does every

20      single amino acid substitution or deletion with in Aβ1-5 necessarily significantly affect
binding affinity. Epitope specificity of an antibody can be determined, for example, by
forming a phage display library in which different members display different
subsequences of Aβ. The phage display library is then selected for members specifically
binding to an antibody under test. A family of sequences is isolated. Typically, such a

25      family contains a common core sequence, and varying lengths of flanking sequences in
different members. The shortest core sequence showing specific binding to the antibody
defines the epitope bound by the antibody. Antibodies can also be tested for epitope
specificity in a competition assay with an antibody whose epitope specificity has already
been determined. For example, antibodies that compete with the 3D6 antibody for

30      binding to Aβ bind to the same or similar epitope as 3D6, i.e., within residues Aβ 1-5.
Likewise antibodies that compete with the 10D5 antibody bind to the same or similar
epitope, i.e., within residues Aβ 3-7. Screening antibodies for epitope specificity is a

useful predictor of therapeutic efficacy. For example, an antibody determined to bind to an epitope within residues 1-7 of Aβ is likely to be effective in preventing and treating Alzheimer's disease according to the methodologies of the present invention.

Monoclonal or polyclonal antibodies that specifically bind to a preferred

5 segment of Aβ without binding to other regions of Aβ have a number of advantages relative to monoclonal antibodies binding to other regions or polyclonal sera to intact Aβ. First, for equal mass dosages, dosages of antibodies that specifically bind to preferred segments contain a higher molar dosage of antibodies effective in clearing amyloid plaques. Second, antibodies specifically binding to preferred segments can

10 induce a clearing response against amyloid deposits without inducing a clearing response against intact APP polypeptide, thereby reducing the potential side effects.

### _1. Production of Nonhuman Antibodies_

The present invention features non-human antibodies, for example,

15 antibodies having specificity for the preferred Aβ epitopes of the invention. Such antibodies can be used in formulating various therapeutic compositions of the invention or, preferably, provide complementarity determining regions for the production of humanized or chimeric antibodies (described in detail below). The production of non-human monoclonal antibodies, _e.g._, murine, guinea pig, primate, rabbit or rat, can be

20 accomplished by, for example, immunizing the animal with Aβ. A longer polypeptide comprising Aβ or an immunogenic fragment of Aβ or anti-idiotypic antibodies to an antibody to Aβ can also be used. See Harlow & Lane, _supra_, incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis or by recombinant expression. Optionally, the immunogen can be

25 administered fused or otherwise complexed with a carrier protein, as described below. Optionally, the immunogen can be administered with an adjuvant. The term "adjuvant" refers to a compound that when administered in conjunction with an antigen augments the immune response to the antigen, but when administered alone does not generate an immune response to the antigen. Adjuvants can augment an immune response by

30 several mechanisms including lymphocyte recruitment, stimulation of B and/or T cells, and stimulation of macrophages. Several types of adjuvant can be used as described

below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals.

Rabbits or guinea pigs are typically used for making polyclonal antibodies. Exemplary preparation of polyclonal antibodies, *e.g.*, for passive protection, can be performed as follows. 125 non-transgenic mice are immunized with 100 μg Aβ1-42, plus CFA/IFA adjuvant, and euthanized at 4-5 months. Blood is collected from immunized mice. IgG is separated from other blood components. Antibody specific for the immunogen may be partially purified by affinity chromatography. An average of about 0.5-1 mg of immunogen-specific antibody is obtained per mouse, giving a total of 60-120 mg.

Mice are typically used for making monoclonal antibodies. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of Aβ into a mouse, preparing hybridomas and screening the hybridomas for an antibody that specifically binds to Aβ. Optionally, antibodies are screened for binding to a specific region or desired fragment of Aβ without binding to other nonoverlapping fragments of Aβ. The latter screening can be accomplished by determining binding of an antibody to a collection of deletion mutants of an Aβ peptide and determining which deletion mutants bind to the antibody. Binding can be assessed, for example, by Western blot or ELISA. The smallest fragment to show specific binding to the antibody defines the epitope of the antibody. Alternatively, epitope specificity can be determined by a competition assay is which a test and reference antibody compete for binding to Aβ. If the test and reference antibodies compete, then they bind to the same epitope or epitopes sufficiently proximal such that binding of one antibody interferes with binding of the other. The preferred isotype for such antibodies is mouse isotype IgG2a or equivalent isotype in other species. Mouse isotype IgG2a is the equivalent of human isotype IgG1.

### 2. *Chimeric and Humanized Antibodies*

The present invention also features chimeric and/or humanized antibodies (*i.e.*, chimeric and/or humanized immunoglobulins) specific for beta amyloid peptide. Chimeric and/or humanized antibodies have the same or similar binding specificity and affinity as a mouse or other nonhuman antibody that provides the starting material for construction of a chimeric or humanized antibody.

A. Production of Chimeric Antibodies

The term "chimeric antibody" refers to an antibody whose light and heavy chain genes have been constructed, typically by genetic engineering, from

5   immunoglobulin gene segments belonging to different species. For example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments, such as IgG1 and IgG4. Human isotype IgG1 is preferred. A typical chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C or effector domain from a

10  human antibody.

B. Production of Humanized Antibodies

The term "humanized antibody" refers to an antibody comprising at least one chain comprising variable region framework residues substantially from a human

15  antibody chain (referred to as the acceptor immunoglobulin or antibody) and at least one
·   complementarity determining region substantially from a mouse-antibody, (referred to as the donor immunoglobulin or antibody). See, Queen et al., Proc. Natl. Acad. Sci. USA 86:10029-10033 (1989), US 5,530,101, US 5,585,089, US 5,693,761, US 5,693,762, Selick et al., WO 90/07861, and Winter, US 5,225,539 (incorporated by

20  reference in their entirety for all purposes). The constant region(s), if present, are also substantially or entirely from a human immunoglobulin.

The substitution of mouse CDRs into a human variable domain framework is most likely to result in retention of their correct spatial orientation if the human variable domain framework adopts the same or similar conformation to the

25  mouse variable framework from which the CDRs originated. This is achieved by obtaining the human variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable framework domains from which the CDRs were derived. The heavy and light chain variable framework regions can be derived from the same or different human antibody

30  sequences. The human antibody sequences can be the sequences of naturally occurring human antibodies or can be consensus sequences of several human antibodies. See

Kettleborough *et al.*, *Protein Engineering* 4:773 (1991); Kolbinger *et al.*, *Protein Engineering* 6:971 (1993) and Carter *et al.*, WO 92/22653.

Having identified the complementarity determining regions of the murine donor immunoglobulin and appropriate human acceptor immunoglobulins, the next step

5    is to determine which, if any, residues from these components should be substituted to optimize the properties of the resulting humanized antibody. In general, substitution of human amino acid residues with murine should be minimized, because introduction of murine residues increases the risk of the antibody eliciting a human-anti-mouse-antibody (HAMA) response in humans. Art-recognized methods of determining immune

10   response can be performed to monitor a HAMA response in a particular patient or during clinical trials. Patients administered humanized antibodies can be given an immunogenicity assessment at the beginning and throughout the administration of said therapy. The HAMA response is measured, for example, by detecting antibodies to the humanized therapeutic reagent, in serum samples from the patient using a method

15   known to one in the art, including surface plasmon resonance technology (BIACORE) and/or solid-phase ELISA analysis.

Certain amino acids from the human variable region framework residues are selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. The unnatural juxtaposition of murine CDR regions with

20   human variable framework region can result in unnatural conformational restraints, which, unless corrected by substitution of certain amino acid residues, lead to loss of binding affinity.

The selection of amino acid residues for substitution is determined, in part, by computer modeling. Computer hardware and software are described herein for

25   producing three-dimensional images of immunoglobulin molecules. In general, molecular models are produced starting from solved structures for immunoglobulin chains or domains thereof. The chains to be modeled are compared for amino acid sequence similarity with chains or domains of solved three-dimensional structures, and the chains or domains showing the greatest sequence similarity is/are selected as starting

30   points for construction of the molecular model. Chains or domains sharing at least 50% sequence identity are selected for modeling, and preferably those sharing at least 60%, 70%, 80%, 90% sequence identity or more are selected for modeling. The solved

starting structures are modified to allow for differences between the actual amino acids in the immunoglobulin chains or domains being modeled, and those in the starting structure. The modified structures are then assembled into a composite immunoglobulin. Finally, the model is refined by energy minimization and by verifying

5    that all atoms are within appropriate distances from one another and that bond lengths and angles are within chemically acceptable limits.

The selection of amino acid residues for substitution can also be determined, in part, by examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of

10   particular amino acids. For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid should usually be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

15                          (1)    noncovalently binds antigen directly,

                            (2)    is adjacent to a CDR region,

                            (3)    otherwise interacts with a CDR region (e.g., is within about 3-6 Å of a CDR region as determined by computer modeling), or

                            (4)    participates in the VL-VH interface.

20   Residues which "noncovalently bind antigen directly" include amino acids in positions in framework regions which are have a good probability of directly  ·  interacting with amino acids on the antigen according to established chemical forces, for example, by hydrogen bonding, Van der Waals forces, hydrophobic interactions, and the like.

25   CDR and framework regions are as defined by Kabat et al. or Chothia et al., supra. When framework residues, as defined by Kabat et al., supra, constitute structural loop residues as defined by Chothia et al., supra, the amino acids present in the mouse antibody may be selected for substitution into the humanized antibody. Residues which are "adjacent to a CDR region" include amino acid residues in positions

30   immediately adjacent to one or more of the CDRs in the primary sequence of the humanized immunoglobulin chain, for example, in positions immediately adjacent to a CDR as defined by Kabat, or a CDR as defined by Chothia (See e.g., Chothia and Lesk

JMB 196:901 (1987)). These amino acids are particularly likely to interact with the amino acids in the CDRs and, if chosen from the acceptor, to distort the donor CDRs and reduce affinity. Moreover, the adjacent amino acids may interact directly with the antigen (Amit *et al.*, Science, 233:747 (1986), which is incorporated herein by reference)

5   and selecting these amino acids from the donor may be desirable to keep all the antigen contacts that provide affinity in the original antibody.

Residues that "otherwise interact with a CDR region" include those that are determined by secondary structural analysis to be in a spatial orientation sufficient to effect a CDR region. In one embodiment, residues that "otherwise interact with a CDR

10  region" are identified by analyzing a three-dimensional model of the donor immunoglobulin (*e.g.*, a computer-generated model). A three-dimensional model, typically of the original donor antibody, shows that certain amino acids outside of the CDRs are close to the CDRs and have a good probability of interacting with amino acids in the CDRs by hydrogen bonding, Van der Waals forces, hydrophobic interactions, etc.

15  At those amino acid positions, the donor immunoglobulin amino acid rather than the acceptor immunoglobulin amino acid may be selected. Amino acids according to this criterion will generally have a side chain atom within about 3 angstrom units (Å) of some atom in the CDRs and must contain an atom that could interact with the CDR atoms according to established chemical forces, such as those listed above.

20  In the case of atoms that may form a hydrogen bond, the 3 Å is measured between their nuclei, but for atoms that do not form a bond, the 3 Å is measured between their Van der Waals surfaces. Hence, in the latter case, the nuclei must be within about 6 Å (3 Å plus the sum of the Van der Waals radii) for the atoms to be considered capable of interacting. In many cases the nuclei will be from 4 or 5 to 6 Å apart. In determining

25  whether an amino acid can interact with the CDRs, it is preferred not to consider the last 8 amino acids of heavy chain CDR 2 as part of the CDRs, because from the viewpoint of structure, these 8 amino acids behave more as part of the framework.

Amino acids that are capable of interacting with amino acids in the CDRs, may be identified in yet another way. The solvent accessible surface area of each

30  framework amino acid is calculated in two ways: (1) in the intact antibody, and (2) in a hypothetical molecule consisting of the antibody with its CDRs removed. A significant difference between these numbers of about 10 square angstroms or more shows that

access of the framework amino acid to solvent is at least partly blocked by the CDRs, and therefore that the amino acid is making contact with the CDRs. Solvent accessible surface area of an amino acid may be calculated based on a three-dimensional model of an antibody, using algorithms known in the art (*e.g.*, Connolly, J. Appl. Cryst. 16:548

5      (1983) and Lee and Richards, J. Mol. Biol. 55:379 (1971), both of which are incorporated herein by reference). Framework amino acids may also occasionally interact with the CDRs indirectly, by affecting the conformation of another framework amino acid that in turn contacts the CDRs.

The amino acids at several positions in the framework are known to be

10     capable of interacting with the CDRs in many antibodies (Chothia and Lesk, *supra*, Chothia *et al.*, *supra* and Tramontano *et al.*, J. Mol. Biol. 215:175 (1990), all of which are incorporated herein by reference). Notably, the amino acids at positions 2, 48, 64 and 71 of the light chain and 26-30, 71 and 94 of the heavy chain (numbering according to Kabat) are known to be capable of interacting with the CDRs in many antibodies.

15     The amino acids at positions 35 in the light chain and 93 and 103 in the heavy chain are also likely to interact with the CDRs. At all these numbered positions, choice of the donor amino acid rather than the acceptor amino acid (when they differ) to be in the humanized immunoglobulin is preferred. On the other hand, certain residues capable of interacting with the CDR region, such as the first 5 amino acids of the light chain, may

20     sometimes be chosen from the acceptor immunoglobulin without loss of affinity in the humanized immunoglobulin.

Residues which "participate in the VL-VH interface" or "packing residues" include those residues at the interface between VL and VH as defined, for example, by Novotny and Haber, Proc. Natl. Acad. Sci. USA, 82:4592-66 (1985) or

25     Chothia *et al*, *supra*. Generally, unusual packing residues should be retained in the humanized antibody if they differ from those in the human frameworks.

In general, one or more of the amino acids fulfilling the above criteria is substituted. In some embodiments, all or most of the amino acids fulfilling the above criteria are substituted. Occasionally, there is some ambiguity about whether a

30     particular amino acid meets the above criteria, and alternative variant immunoglobulins are produced, one of which has that particular substitution, the other of which does not.

Alternative variant immunoglobulins so produced can be tested in any of the assays described herein for the desired activity, and the preferred immunoglobulin selected.

Usually the CDR regions in humanized antibodies are substantially identical, and more usually, identical to the corresponding CDR regions of the donor antibody. Although not usually desirable, it is sometimes possible to make one or more conservative amino acid substitutions of CDR residues without appreciably affecting the binding affinity of the resulting humanized immunoglobulin. By conservative substitutions is intended combinations such as gly, ala; val, ile, leu; asp, glu; asn, gln; ser, thr; lys, arg; and phe, tyr.

Additional candidates for substitution are acceptor human framework amino acids that are unusual or "rare" for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins. For example, substitution may be desirable when the amino acid in a human framework region of the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is common for that position in human immunoglobulin sequences; or when the amino acid in the acceptor immunoglobulin is rare for that position and the corresponding amino acid in the donor immunoglobulin is also rare, relative to other human sequences. These criterion help ensure that an atypical amino acid in the human framework does not disrupt the antibody structure. Moreover, by replacing an unusual human acceptor amino acid with an amino acid from the donor antibody that happens to be typical for human antibodies, the humanized antibody may be made less immunogenic.

The term "rare", as used herein, indicates an amino acid occurring at that position in less than about 20% but usually less than about 10% of sequences in a representative sample of sequences, and the term "common", as used herein, indicates an amino acid occurring in more than about 25% but usually more than about 50% of sequences in a representative sample. For example, all human light and heavy chain variable region sequences are respectively grouped into "subgroups" of sequences that are especially homologous to each other and have the same amino acids at certain critical positions (Kabat *et al., supra*). When deciding whether an amino acid in a human acceptor sequence is "rare" or "common" among human sequences, it will often

29

be preferable to consider only those human sequences in the same subgroup as the acceptor sequence.

Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the alternative definition proposed by Chothia *et al., supra*. Additional candidates for substitution are acceptor human framework amino acids that would be identified as part of a CDR region under the AbM and/or contact definitions. Notably, CDR1 in the variable heavy chain is defined as including residues 26-32.

Additional candidates for substitution are acceptor framework residues that correspond to a rare or unusual donor framework residue. Rare or unusual donor framework residues are those that are rare or unusual (as defined herein) for murine antibodies at that position. For murine antibodies, the subgroup can be determined according to Kabat and residue positions identified which differ from the consensus. These donor specific differences may point to somatic mutations in the murine sequence which enhance activity. Unusual residues that are predicted to affect binding are retained, whereas residues predicted to be unimportant for binding can be substituted.

Additional candidates for substitution are non-germline residues occurring in an acceptor framework region. For example, when an acceptor antibody chain (*i.e.*, a human antibody chain sharing significant sequence identity with the donor antibody chain) is aligned to a germline antibody chain (likewise sharing significant sequence identity with the donor chain), residues not matching between acceptor chain framework and the germline chain framework can be substituted with corresponding residues from the germline sequence.

Other than the specific amino acid substitutions discussed above, the framework regions of humanized immunoglobulins are usually substantially identical, and more usually, identical to the framework regions of the human antibodies from which they were derived. Of course, many of the amino acids in the framework region make little or no direct contribution to the specificity or affinity of an antibody. Thus, many individual conservative substitutions of framework residues can be tolerated without appreciable change of the specificity or affinity of the resulting humanized immunoglobulin. Thus, in one embodiment the variable framework region of the humanized immunoglobulin shares at least 85% sequence identity to a human variable

framework region sequence or consensus of such sequences. In another embodiment, the variable framework region of the humanized immunoglobulin shares at least 90%, preferably 95%, more preferably 96%, 97%, 98% or 99% sequence identity to a human variable framework region sequence or consensus of such sequences. In general,

5        however, such substitutions are undesirable.

The humanized antibodies preferably exhibit a specific binding affinity for antigen of at least $10^7$, $10^8$, $10^9$ or $10^{10}$ M$^{-1}$. Usually the upper limit of binding affinity of the humanized antibodies for antigen is within a factor of three, four or five of that of the donor immunoglobulin. Often the lower limit of binding affinity is also

10       within a factor of three, four or five of that of donor immunoglobulin. Alternatively, the binding affinity can be compared to that of a humanized antibody having no substitutions (e.g., an antibody having donor CDRs and acceptor FRs, but no FR substitutions). In such instances, the binding of the optimized antibody (with substitutions) is preferably at least two- to three-fold greater, or three- to four-fold

15       greater, than that of the unsubstituted antibody. For making comparisons, activity of the various antibodies can be determined, for example, by BIACORE (i.e., surface plasmon resonance using unlabelled reagents) or competitive binding assays.


### C. Production of Humanized 3D6 Antibodies

20       A preferred embodiment of the present invention features a humanized antibody to the N-terminus of Aβ, in particular, for use in the therapeutic and/or diagnostic methodologies described herein. A particularly preferred starting material for production of humanized antibodies is 3D6. 3D6 is specific for the N-terminus of Aβ and has been shown to mediate phagocytosis (e.g., induce phagocytosis) of amyloid

25       plaque (see Examples I-V). The cloning and sequencing of cDNA encoding the 3D6 antibody heavy and light chain variable regions is described in Example VI.

Suitable human acceptor antibody sequences are identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies. The comparison is performed separately for

30       heavy and light chains but the principles are similar for each. In particular, variable domains from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine VL and VH framework regions were identified by

query of the Kabat Database using NCBI BLAST (publicly accessible through the National Institutes of Health NCBI internet server) with the respective murine framework sequences. In one embodiment, acceptor sequences sharing greater that 50% sequence identity with murine donor sequences are selected. Preferably, acceptor

5    antibody sequences sharing 60%, 70%, 80%, 90% or more are selected.

A computer comparison of 3D6 revealed that the 3D6 light chain shows the greatest sequence identity to human light chains of subtype kappa II, and that the 3D6 heavy chain shows greatest sequence identity to human heavy chains of subtype III, as defined by Kabat *et al.*, *supra*. Thus, light and heavy human framework regions are

10   preferably derived from human antibodies of these subtypes, or from consensus sequences of such subtypes. The preferred light chain human variable regions showing greatest sequence identity to the corresponding region from 3D6 are from antibodies having Kabat ID Numbers 019230, 005131, 005058, 005057, 005059, U21040 and U41645, with 019230 being more preferred. The preferred heavy chain human variable

15   regions showing greatest sequence identity to the corresponding region from 3D6 are from antibodies having Kabat ID Numbers 045919, 000459, 000553, 000386 and M23691, with 045919 being more preferred.

Residues are next selected for substitution, as follows. When an amino acid differs between a 3D6 variable framework region and an equivalent human variable

20   framework region, the human framework amino acid should usually be substituted by the equivalent mouse amino acid if it is reasonably expected that the amino acid:

(1) noncovalently binds antigen directly,

(2) is adjacent to a CDR region, is part of a CDR region under the alternative definition proposed by Chothia *et al.*, *supra*, or otherwise interacts with a

25   CDR region (*e.g.*, is within about 3A of a CDR region) (*e.g.*, amino acids at positions L2, H49 and H94 of 3D6), or

(3) participates in the VL-VH interface (*e.g.*, amino acids at positions L36, L46 and H93 of 3D6).

Computer modeling of the 3D6 antibody heavy and light chain variable

30   regions, and humanization of the 3D6 antibody is described in Example VII. Briefly, a three-dimensional model was generated based on the closest solved murine antibody structures for the heavy and light chains. For this purpose, an antibody designated 1CR9

(Protein Data Bank (PDB) ID: 1CR9, Kanyo *et al., J. Mol. Biol.* 293:855 (1999)) was

chosen as a template for modeling the 3D6 light chain, and an antibody designated

1OPG (PDB ID: 1OPG, Kodandapani *et al., J. Biol. Chem.* 270:2268 (1995)) was

chosen as the template for modeling the heavy chain. The model was further refined by

5     a series of energy minimization steps to relieve unfavorable atomic contacts and

optimize electrostatic and van der Walls interactions. The solved structure of 1qkz

(PDB ID: 1QKZ, Derrick *et al., J. Mol. Biol.* 293:81 (1999)) was chosen as a template

for modeling CDR3 of the heavy chain as 3D6 and 1OPG did not exhibit significant

sequence homology in this region when aligned for comparison purposes.

10              Three-dimensional structural information for the antibodies described

herein is publicly available, for example, from the Research Collaboratory for Structural

Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible *via* the World

Wide Web internet and is described by Berman *et al.* (2000) *Nucleic Acids Research,*

28:235. Computer modeling allows for the identification of CDR-interacting residues.

15    The computer model of the structure of 3D6 can in turn serve as a starting point for

predicting the three-dimensional structure of an antibody containing the 3D6

complementarity determining regions substituted in human framework structures.

Additional models can be constructed representing the structure as further amino acid

substitutions are introduced.

20              In general, substitution of one, most or all of the amino acids fulfilling the

above criteria is desirable. Accordingly, the humanized antibodies of the present

invention will usually contain a substitution of a human light chain framework residue

with a corresponding 3D6 residue in at least 1, 2 or 3, and more usually 4, of the

following positions: L1, L2, L36 and L46. The humanized antibodies also usually

25    contain a substitution of a human heavy chain framework residue with a corresponding

3D6 residue in at least 1, 2, and sometimes 3, of the following positions: H49, H93 and

H94. Humanized antibodies can also contain a substitution of a heavy chain framework

residue with a corresponding germline residue in at least 1, 2, and sometimes 3, of the

following positions: H74, H77 and H89.

30              Occasionally, however, there is some ambiguity about whether a

particular amino acid meets the above criteria, and alternative variant immunoglobulins

are produced, one of which has that particular substitution, the other of which does not.

In instances where substitution with a murine residue would introduce a residue that is rare in human immunoglobulins at a particular position, it may be desirable to test the antibody for activity with or without the particular substitution. If activity (*e.g.*, binding affinity and/or binding specificity) is about the same with or without the substitution, the antibody without substitution may be preferred, as it would be expected to elicit less of a HAHA response, as described herein.

Other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of more typical human immunoglobulins. Alternatively, amino acids from equivalent positions in the mouse 3D6 can be introduced into the human framework regions when such amino acids are typical of human immunoglobulin at the equivalent positions.

In additional embodiments, when the human light chain framework acceptor immunoglobulin is Kabat ID Number 019230, the light chain contains substitutions in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12 or more usually 13, of the following positions: L7, L10, L12, L15, L17, L39, L45, L63, L78, L83, L85, L100 or L104. In additional embodiments when the human heavy chain framework acceptor immunoglobulin is Kabat ID Number 045919, the heavy chain contains substitutions in at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or more usually 15, of the following positions: H3, H5, H13, H16, H19, H40, H41, H42, H44, H72, H77, H82A, H83, H84, or H108. These positions are substituted with the amino acid from the equivalent position of a human immunoglobulin having a more typical amino acid residue. Examples of appropriate amino acids to substitute are shown in Figures 1 and 2.

Other candidates for substitution are non-germline residues occurring in a framework region. A computer comparison of 3D6 with known germline sequences revealed that heavy chains showing the greatest degree of sequence identity include germline variable region sequences VH3-48, VH3-23, VH3-7, VH3-21 and VH3-11, with VH3-23 being more preferred. Alignment of Kabat ID 045919 with VH3-23 reveals that residues H74, H77 and/or H89 may be selected for substitution with corresponding germline residues (*e.g.*, residues H74, H77 and/or H89 when comparing Kabat ID 045919 and VH3-23). Likewise, germline sequences having the greatest degree of identity to the 3D6 light chain include A1, A17, A18, A2 and A19, with A19

being most preferred. Residues not matching between a selected light chain acceptor framework and one of these germline sequences could be selected for substitution with the corresponding germline residue.

5      Table 1 summarizes the sequence analysis of the 3D6 VH and VL regions. Additional mouse and human structures that can be used for computer modeling of the 3D6 antibody and additional human antibodies are set forth as well as germline sequences that can be used in selecting amino acid substitutions. Rare mouse residues are also set forth in Table 1. Rare mouse residues are identified by comparing the donor VL and/or VH sequences with the sequences of other members of the

10     subgroup to which the donor VL and/or VH sequences belong (according to Kabat) and identifying the residue positions which differ from the consensus. These donor specific differences may point to somatic mutations which enhance activity. Unusual or rare residues close to the binding site may possibly contact the antigen, making it desirable to retain the mouse residue. However, if the unusual mouse residue is not important for

15     binding, use of the corresponding acceptor residue is preferred as the mouse residue may create immunogenic neoepitopes in the humanized antibody. In the situation where an unusual residue in the donor sequence is actually a common residues in the corresponding acceptor sequence, the preferred residue is clearly the acceptor residue.

20     Table 1: Summary of 3D6 V-region sequence

| Chain | Heavy | Light |
|---|---|---|
| Mouse subgroup (Kabat seq ID#) | IIID (002688) | II (005840-005844, 005851-005853, 005857, 005863) |
| Mouse homologs (Kabat/Genbank) | 002727/163.1'CL 002711/H35-C6'CL 002733/8-1-12-5-3-1(A2-1)'CL 002715/ASWA2'CL 020669/#14'CL | 005840/1210.7 005843/42.4b.12.2'CL 005842/BXW-14'CL 005841/42.7B3.2'CL 005851/36-60CRI- |
| Rare amino acids (% frequency of occurrence in class) | N40 (0.233%) D42 (0.699%) | Y1(.035%) I15 (3.3%) D27 (0.867%)-CDR1 I78 (0.677%) L85 (0.625%) W89 (0.815%)-CDR3 |

|  |  | K106A (0.295%) |
|---|---|---|
|  |  |  |
| Human Subgroup | III (000488-000491, 000503, 000624) | II (005046) |
| Chothia canonical CDR groupings [pdb example] | H1: class 1 [2fbj] <br> H2: class 3 [1igc] | L1: class 4 [1rmf] <br> L2: class1 [1lmk] <br> L3: class 1 [1tet] |
| Closest solved mouse structures | PDB ID: 1OPG Kodandapani *et al., supra*; (72% 2Å) | PDB ID: 1CR9; Kanyo *et al., supra*; (94%, 2Å) <br> PDB ID: 1NLD; Davies *et al., Acta Crystallogr. D. Biol. Crystallog.* 53:186 (1997); (98%, 2.8Å) |
| Closest solved human structures | 1VH (68%, nmr) <br> 443560 (65%, IgG, λ myeloma, 1.8Å) <br> KOL/2FB4H (60%, myeloma, 3Å) | 1LVE (57%, LEN) <br> 1B6DA (54%, B-J dimer, 2.8Å); <br> 1VGEL (54%, autoAb) |
| Germline query (Hu) results (top 4) | VH3-48 (4512283/BAA75032.1) <br> VH3-23 (4512287/BAA75046.1) <br> VH3-7 (4512300/BAA75056.1) <br> VH3-21 (4512287/BAA75047.1) <br> VH3-11 (4152300/BAA75053.1) | A1(x63402) <br> A17 (x63403) <br> A18 (X63396) <br> A2 (m31952) <br> A19 (x63397) |

*heavy chain and light chain from the same antibody (O-81, Hirabayashi *et al.* NAR 20:2601).

Kabat ID sequences referenced herein are publicly available, for example, from the Northwestern University Biomedical Engineering Department's Kabat Database of Sequences of Proteins of Immunological Interest. Three-dimensional structural information for antibodies described herein is publicly available, for example, from the Research Collaboratory for Structural Bioinformatics' Protein Data Bank (PDB). The PDB is freely accessible *via* the World Wide Web internet and is described by Berman *et al.* (2000) *Nucleic Acids Research*, p235-242. Germline gene sequences referenced herein are publicly available, for example, from the National Center for Biotechnology Information (NCBI) database of sequences in collections of Igh, Ig kappa and Ig lambda germline V genes (as a division of the National Library of Medicine (NLM) at the National Institutes of Health (NIH)). Homology searching of the NCBI "Ig Germline Genes" database is provided by IgG BLAST™.

In a preferred embodiment, a humanized antibody of the present invention contains (i) a light chain comprising a variable domain comprising murine 3D6 VL CDRs and a human acceptor framework, the framework having at least one, preferably two, three or four residues selected from the group consisting of L1, L2, L36,

5      and L46 substituted with the corresponding 3D6 residue and (ii) a heavy chain comprising 3D6 VH CDRs and a human acceptor framework, the framework having at least one, preferably two or three residues selected from the group consisting of H49, H93 and H94 substituted with the corresponding 3D6 residue, and, optionally, at least one, preferably two or three residues selected from the group consisting of H74, H77

10     and H89 is substituted with a corresponding human germline residue..

In a more preferred embodiment, a humanized antibody of the present invention contains (i) a light chain comprising a variable domain comprising murine 3D6 VL CDRs and a human acceptor framework, the framework having residue 1 substituted with a tyr (Y), residue 2 substituted with a val (V), residue 36 substituted

15     with a leu (L) and/or residue 46 substituted with an arg (R), and (ii) a heavy chain comprising 3D6 VH CDRs and a human acceptor framework, the framework having residue 49 substituted with an ala (A), residue 93 substituted with a val (V) and/or residue 94 substituted with an arg (R), and, optionally, having residue 74 substituted with a ser (S), residue 77 substituted with a thr (T) and/or residue 89 substituted with a

20     val (V).

In a particularly preferred embodiment, a humanized antibody of the present invention has structural features, as described herein, and further has at least one (preferably two, three, four or all) of the following activities: (1) binds aggregated Aβ1-42 (e.g., as determined by ELISA); (2) binds Aβ in plaques (e.g., staining of AD and/or

25     PDAPP plaques); (3) binds Aβ with two- to three- fold higher binding affinity as compared to chimeric 3D6 (e.g., 3D6 having murine CDRs and human acceptor FRs); (4) mediates phagocytosis of Aβ (e.g., in an ex vivo phagocytosis assay, as described herein); and (5) crosses the blood-brain barrier (e.g., demonstrates short-term brain localization, for example, in a PDAPP animal model, as described herein).

30     In another embodiment, a humanized antibody of the present invention has structural features, as described herein, binds Aβ in a manner or with an affinity sufficient to elicit at least one of the following in vivo effects: (1) reduce Aβ plaque

37

burden; (2) prevent plaque formation; (3) reduce levels of soluble Aβ; (4) reduce the neuritic pathology associated with an amyloidogenic disorder; (5) lessens or ameliorate at least one physiological symptom associated with an amyloidogenic disorder; and/or (6) improves cognitive function.

5        In another embodiment, a humanized antibody of the present invention has structural features, as described herein, and specifically binds to an epitope comprising residues 1-5 or 3-7 of Aβ.


### 3. Human Antibodies

10       Human antibodies against Aβ are provided by a variety of techniques described below. Some human antibodies are selected by competitive binding experiments, or otherwise, to have the same epitope specificity as a particular mouse antibody, such as one of the mouse monoclonals described herein. Human antibodies can also be screened for a particular epitope specificity by using only a fragment of Aβ
15    as the immunogen, and/or by screening antibodies against a collection of deletion mutants of Aβ. Human antibodies preferably have human IgG1 isotype specificity.

a. Trioma Methodology

The basic approach and an exemplary cell fusion partner, SPAZ-4, for use in this approach have been described by Oestberg *et al.*, Hybridoma 2:361 (1983);
20   Oestberg, US Patent No. 4,634,664; and Engleman *et al.*, US Patent 4,634,666 (each of which is incorporated by reference in its entirety for all purposes). The antibody-producing cell lines obtained by this method are called triomas, because they are descended from three cells; two human and one mouse. Initially, a mouse myeloma line is fused with a human B-lymphocyte to obtain a non-antibody-producing xenogeneic
25    hybrid cell, such as the SPAZ-4 cell line described by Oestberg, *supra*. The xenogeneic cell is then fused with an immunized human B-lymphocyte to obtain an antibody-producing trioma cell line. Triomas have been found to produce antibody more stably than ordinary hybridomas made from human cells.

The immunized B-lymphocytes are obtained from the blood, spleen,
30   lymph nodes or bone marrow of a human donor. If antibodies against a specific antigen or epitope are desired, it is preferable to use that antigen or epitope thereof for immunization. Immunization can be either *in vivo* or *in vitro*. For *in vivo* immunization,

B cells are typically isolated from a human immunized with Aβ, a fragment thereof, larger polypeptide containing Aβ or fragment, or an anti-idiotypic antibody to an antibody to Aβ. In some methods, B cells are isolated from the same patient who is ultimately to be administered antibody therapy. For *in vitro* immunization, B-

5      lymphocytes are typically exposed to antigen for a period of 7-14 days in a media such as RPMI-1640 (see Engleman, *supra*) supplemented with 10% human plasma.

The immunized B-lymphocytes are fused to a xenogeneic hybrid cell such as SPAZ-4 by well-known methods. For example, the cells are treated with 40-50% polyethylene glycol of MW 1000-4000, at about 37 degrees C, for about 5-10 min.

10     Cells are separated from the fusion mixture and propagated in media selective for the desired hybrids (*e.g.*, HAT or AH). Clones secreting antibodies having the required binding specificity are identified by assaying the trioma culture medium for the ability to bind to Aβ or a fragment thereof. Triomas producing human antibodies having the desired specificity are subcloned by the limiting dilution technique and grown *in vitro* in

15     culture medium. The trioma cell lines obtained are then tested for the ability to bind Aβ or a fragment thereof.

Although triomas are genetically stable they do not produce antibodies at very high levels. Expression levels can be increased by cloning antibody genes from the trioma into one or more expression vectors, and transforming the vector into standard

20     mammalian, bacterial or yeast cell lines.

b. Transgenic Non-Human Mammals

Human antibodies against Aβ can also be produced from non-human transgenic mammals having transgenes encoding at least a segment of the human

25     immunoglobulin locus. Usually, the endogenous immunoglobulin locus of such transgenic mammals is functionally inactivated. Preferably, the segment of the human immunoglobulin locus includes unrearranged sequences of heavy and light chain components. Both inactivation of endogenous immunoglobulin genes and introduction of exogenous immunoglobulin genes can be achieved by targeted homologous

30     recombination, or by introduction of YAC chromosomes. The transgenic mammals resulting from this process are capable of functionally rearranging the immunoglobulin component sequences, and expressing a repertoire of antibodies of various isotypes

encoded by human immunoglobulin genes, without expressing endogenous immunoglobulin genes. The production and properties of mammals having these properties are described in detail by, *e.g.*, Lonberg *et al.*, WO93/12227 (1993); US

5      5,877,397, US 5,874,299, US 5,814,318, US 5,789,650, US 5,770,429, US 5,661,016, US 5,633,425, US 5,625,126, US 5,569,825, US 5,545,806, *Nature* 148:1547 (1994), *Nature Biotechnology* 14:826 (1996), Kucherlapati, WO 91/10741 (1991) (each of which is incorporated by reference in its entirety for all purposes). Transgenic mice are particularly suitable. Anti-Aβ antibodies are obtained by immunizing a transgenic nonhuman mammal, such as described by Lonberg or Kucherlapati, *supra,* with Aβ or a

10     fragment thereof. Monoclonal antibodies are prepared by, *e.g.*, fusing B-cells from such mammals to suitable myeloma cell lines using conventional Kohler-Milstein technology. 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 Aβ or other amyloid peptide as an affinity

15     reagent.

### c. Phage Display Methods

A further approach for obtaining human anti-Aβ antibodies is to screen a DNA library from human B cells according to the general protocol outlined by Huse *et*

20     *al.*, *Science* 246:1275-1281 (1989). As described for trioma methodology, such B cells can be obtained from a human immunized with Aβ, fragments, longer polypeptides containing Aβ or fragments or anti-idiotypic antibodies. Optionally, such B cells are obtained from a patient who is ultimately to receive antibody treatment. Antibodies binding to Aβ or a fragment thereof are selected. Sequences encoding such antibodies

25     (or a binding fragments) are then cloned and amplified. The protocol described by Huse is rendered more efficient in combination with phage-display technology. See, *e.g.*, Dower *et al.*, WO 91/17271, McCafferty *et al.*, WO 92/01047, Herzig *et al.*, US 5,877,218, Winter *et al.*, US 5,871,907, Winter *et al.*, US 5,858,657, Holliger *et al.*, US 5,837,242, Johnson *et al.*, US 5,733,743 and Hoogenboom *et al.*, US 5,565,332 (each of

30     which is incorporated by reference in its entirety for all purposes). 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 an Aβ peptide or fragment thereof.

In a variation of the phage-display method, human antibodies having the binding specificity of a selected murine antibody can be produced. See Winter, WO
5    92/20791. In this method, either the heavy or light chain variable region of the selected murine antibody is used as a starting material. If, for example, a light chain variable region is selected as the starting material, a phage library is constructed in which members display the same light chain variable region (*i.e.*, the murine starting material) and a different heavy chain variable region. The heavy chain variable regions are
10   obtained from a library of rearranged human heavy chain variable regions. A phage showing strong specific binding for Aβ (*e.g.*, at least $10^8$ and preferably at least $10^9$ $M^{-1}$) is selected. The human heavy chain variable region from this phage then serves as a starting material for constructing a further phage library. In this library, each phage displays the same heavy chain variable region (*i.e.*, the region identified from the first
15   display library) and a different light chain variable region. The light chain variable regions are obtained from a library of rearranged human variable light chain regions. Again, phage showing strong specific binding for Aβ are selected. These phage display the variable regions of completely human anti-Aβ antibodies. These antibodies usually have the same or similar epitope specificity as the murine starting material.
20

### 4. Production of Variable Regions

Having conceptually selected the CDR and framework components of humanized immunoglobulins, a variety of methods are available for producing such immunoglobulins. Because of the degeneracy of the code, a variety of nucleic acid
25   sequences will encode each immunoglobulin amino acid sequence. The desired nucleic acid sequences can be produced by *de novo* solid-phase DNA synthesis or by PCR mutagenesis of an earlier prepared variant of the desired polynucleotide. Oligonucleotide-mediated mutagenesis is a preferred method for preparing substitution, deletion and insertion variants of target polypeptide DNA. See Adelman *et al.*, DNA
30   2:183 (1983). Briefly, the target polypeptide DNA is altered by hybridizing an oligonucleotide encoding the desired mutation to a single-stranded DNA template. After hybridization, a DNA polymerase is used to synthesize an entire second complementary

strand of the template that incorporates the oligonucleotide primer, and encodes the selected alteration in the target polypeptide DNA.

### 5. Selection of Constant Regions

The variable segments of antibodies produced as described *supra* (*e.g.*, the heavy and light chain variable regions of chimeric, humanized, or human antibodies) are typically linked to at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Human constant region DNA sequences can be isolated in accordance with well known procedures from a variety of human cells, but preferably immortalized B cells (see Kabat *et al.*, *supra*, and Liu *et al.*, WO87/02671) (each of which is incorporated by reference in its entirety for all purposes). Ordinarily, the antibody will contain both light chain and heavy chain constant regions. The heavy chain constant region usually includes CH1, hinge, CH2, CH3, and CH4 regions. The antibodies described herein include antibodies having all types of constant regions, including IgM, IgG, IgD, IgA and IgE, and any isotype, including IgG1, IgG2, IgG3 and IgG4. The choice of constant region depends, in part, whether antibody-dependent complement and/or cellular mediated toxicity is desired. For example, isotopes IgG1 and IgG3 have complement activity and isotypes IgG2 and IgG4 do not. When it is desired that the antibody (*e.g.*, humanized antibody) exhibit cytotoxic activity, the constant domain is usually a complement fixing constant domain and the class is typically IgG1. When such cytotoxic activity is not desirable, the constant domain may be of the IgG2 class. Choice of isotype can also affect passage of antibody into the brain. Human isotype IgG1 is preferred. Light chain constant regions can be lambda or kappa. The humanized antibody may comprise sequences from more than one class or isotype. Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab' F(ab')2, and Fv, or as single chain antibodies in which heavy and light chain variable domains are linked through a spacer.

## 6. Expression of Recombinant Antibodies

Chimeric, humanized and human antibodies are typically produced by recombinant expression. Nucleic acids encoding humanized light and heavy chain variable regions, optionally linked to constant regions, are inserted into expression

5    vectors. The light and heavy chains can be cloned in the same or different expression vectors. The DNA segments encoding immunoglobulin chains are operably linked to control sequences in the expression vector(s) that ensure the expression of immunoglobulin polypeptides. Expression control sequences include, but are not limited to, promoters (*e.g.*, naturally-associated or heterologous promoters), signal

10   sequences, enhancer elements, and transcription termination sequences. Preferably, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been         •
incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and the collection and purification

15   of the crossreacting antibodies.

These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers (*e.g.*, ampicillin-resistance, hygromycin-resistance, tetracycline resistance or neomycin resistance) to permit detection of those

20   cells transformed with the desired DNA sequences (see, *e.g.*, Itakura *et al.*, US Patent 4,704,362).

*E. coli* is one prokaryotic host particularly useful for cloning the polynucleotides (*e.g.*, DNA sequences) of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilus*, and other enterobacteriaceae,

25   such as *Salmonella, Serratia*, and various *Pseudomonas* species. In these prokaryotic hosts, one can also make expression vectors, which will typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (trp) promoter system, a beta-lactamase promoter

30   system, or a promoter system from phage lambda. The promoters will typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation.

Other microbes, such as yeast, are also useful for expression. *Saccharomyces* is a preferred yeast host, with suitable vectors having expression control sequences (*e.g.*, promoters), an origin of replication, termination sequences and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic

5      enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

In addition to microorganisms, mammalian tissue cell culture may also be used to express and produce the polypeptides of the present invention (*e.g.*,

10     polynucleotides encoding immunoglobulins or fragments thereof). See Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y. (1987). Eukaryotic cells are actually preferred, because a number of suitable host cell lines capable of secreting heterologous proteins (*e.g.*, intact immunoglobulins) have been developed in the art, and include CHO cell lines, various Cos cell lines, HeLa cells, preferably, myeloma cell

15     lines, or transformed B-cells or hybridomas. Preferably, the cells are nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (Queen *et al.*, *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences.

20     Preferred expression control sequences are promoters derived from immunoglobulin genes, SV40, adenovirus, bovine papilloma virus, cytomegalovirus and the like. See Co *et al.*, *J. Immunol.* 148:1149 (1992).

Alternatively, antibody-coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent

25     expression in the milk of the transgenic animal (see, *e.g.*, Deboer *et al.*, US 5,741,957, Rosen, US 5,304,489, and Meade *et al.*, US 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

The vectors containing the polynucleotide sequences of interest (*e.g.*, the

30     heavy and light chain encoding sequences and expression control sequences) can be transferred into the host cell by well-known methods, which vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for

prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics or viral-based transfection may be used for other cellular hosts. (See generally Sambrook *et al.*, *Molecular Cloning: A Laboratory Manual* (Cold Spring Harbor Press, 2nd ed., 1989) (incorporated by reference in its entirety for all purposes). Other methods

5      used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (see generally, Sambrook *et al.*, *supra*). For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

10               When heavy and light chains are cloned on separate expression vectors, the vectors are co-transfected to obtain expression and assembly of intact immunoglobulins. Once expressed, the whole antibodies, their dimers, individual light and heavy chains, or other immunoglobulin forms of the present invention can be purified according to standard procedures of the art, including ammonium sulfate

15     precipitation, affinity columns, column chromatography, HPLC purification, gel electrophoresis and the like (see generally Scopes, Protein Purification (Springer-Verlag, N.Y., (1982)). Substantially pure immunoglobulins of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity most preferred, for pharmaceutical uses.

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               *7. Antibody Fragments*

               Also contemplated within the scope of the instant invention are antibody fragments. In one embodiment, fragments of non-human, chimeric and/or human antibodies are provided. In another embodiment, fragments of humanized antibodies are

25     provided. Typically, these fragments exhibit specific binding to antigen with an affinity of at least $10^7$, and more typically $10^8$ or $10^9 M^{-1}$. Humanized antibody fragments include separate heavy chains, light chains Fab, Fab' F(ab')2, Fabc, and Fv. Fragments are produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins.

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*8.  Testing Antibodies for Therapeutic Efficacy in Animal Models*

Groups of 7-9 month old PDAPP mice each are injected with 0.5 mg in
PBS of polyclonal anti-Aβ or specific anti-Aβ monoclonal antibodies.  All antibody
preparations are purified to have low endotoxin levels.  Monoclonals can be prepared
5    against a fragment by injecting the fragment or longer form of Aβ into a mouse,
preparing hybridomas and screening the hybridomas for an antibody that specifically
binds to a desired fragment of Aβ without binding to other nonoverlapping fragments of
Aβ.

Mice are injected intraperitoneally as needed over a 4 month period to
10   maintain a circulating antibody concentration measured by ELISA titer of greater than
1/1000 defined by ELISA to Aβ42 or other immunogen.  Titers are monitored and mice
are euthanized at the end of 6 months of injections.  Histochemistry, Aβ levels and
toxicology are performed post mortem.  Ten mice are used per group.


15                            *9.  Screening Antibodies for Clearing Activity*

The invention also provides methods of screening an antibody for activity
in clearing an amyloid deposit or any other antigen, or associated biological entity, for
which clearing activity is desired.  To screen for activity against an amyloid deposit, a
tissue sample from a brain of a patient with Alzheimer's disease or an animal model
20   having characteristic Alzheimer's pathology is contacted with phagocytic cells bearing
an Fc receptor, such as microglial cells, and the antibody under test in a medium *in vitro*.
The phagocytic cells can be a primary culture or a cell line, such as BV-2, C8-B4, or
THP-1.  In some methods, the components are combined on a microscope slide to
facilitate microscopic monitoring.  In some methods, multiple reactions are performed in
25   parallel in the wells of a microtiter dish.  In such a format, a separate miniature
microscope slide can be mounted in the separate wells, or a nonmicroscopic detection
format, such as ELISA detection of Aβ can be used.  Preferably, a series of
measurements is made of the amount of amyloid deposit in the *in vitro* reaction mixture,
starting from a baseline value before the reaction has proceeded, and one or more test
30   values during the reaction.  The antigen can be detected by staining, for example, with a
fluorescently labeled antibody to Aβ or other component of amyloid plaques.  The
antibody used for staining may or may not be the same as the antibody being tested for

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clearing activity. A reduction relative to baseline during the reaction of the amyloid deposits indicates that the antibody under test has clearing activity. Such antibodies are likely to be useful in preventing or treating Alzheimer's and other amyloidogenic diseases.

5          Analogous methods can be used to screen antibodies for activity in clearing other types of biological entities. The assay can be used to detect clearing activity against virtually any kind of biological entity. Typically, the biological entity has some role in human or animal disease. The biological entity can be provided as a tissue sample or in isolated form. If provided as a tissue sample, the tissue sample is

10      preferably unfixed to allow ready access to components of the tissue sample and to avoid perturbing the conformation of the components incidental to fixing. Examples of tissue samples that can be tested in this assay include cancerous tissue, precancerous tissue, tissue containing benign growths such as warts or moles, tissue infected with pathogenic microorganisms, tissue infiltrated with inflammatory cells, tissue bearing

15      pathological matrices between cells (e.g., fibrinous pericarditis), tissue bearing aberrant antigens, and scar tissue. Examples of isolated biological entities that can be used include Aβ, viral antigens or viruses, proteoglycans, antigens of other pathogenic microorganisms, tumor antigens, and adhesion molecules. Such antigens can be obtained from natural sources, recombinant expression or chemical synthesis, among

20      other means. The tissue sample or isolated biological entity is contacted with phagocytic cells bearing Fc receptors, such as monocytes or microglial cells, and an antibody to be tested in a medium. The antibody can be directed to the biological entity under test or to an antigen associated with the entity. In the latter situation, the object is to test whether the biological entity is vicariously phagocytosed with the antigen.

25      Usually, although not necessarily, the antibody and biological entity (sometimes with an associated antigen), are contacted with each other before adding the phagocytic cells. The concentration of the biological entity and/or the associated antigen remaining in the medium, if present, is then monitored. A reduction in the amount or concentration of antigen or the associated biological entity in the medium indicates the antibody has a

30      clearing response against the antigen and/or associated biological entity in conjunction with the phagocytic cells (see, e.g., Example IV).

B. Nucleic Acid Encoding Immunologic and Therapeutic Agents

Immune responses against amyloid deposits can also be induced by administration of nucleic acids encoding antibodies and their component chains used for passive immunization. Such nucleic acids can be DNA or RNA. A nucleic acid segment encoding an immunogen is typically linked to regulatory elements, such as a promoter and enhancer, that allow expression of the DNA segment in the intended target cells of a patient. For expression in blood cells, as is desirable for induction of an immune response, promoter and enhancer elements from light or heavy chain immunoglobulin genes or the CMV major intermediate early promoter and enhancer are suitable to direct expression. The linked regulatory elements and coding sequences are often cloned into a vector. For administration of double-chain antibodies, the two chains can be cloned in the same or separate vectors.

A number of viral vector systems are available including retroviral systems *(see, e.g.,* Lawrie and Tumin, *Cur. Opin. Genet. Develop.* 3:102-109 (1993)); adenoviral vectors *(see, e.g.,* Bett *et al., J. Virol.* 67:5911 (1993)); adeno-associated virus vectors (see, *e.g.,* Zhou *et al., J. Exp. Med.* 179:1867 (1994)), viral vectors from the pox family including vaccinia virus and the avian pox viruses, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest Viruses *(see, e.g.,* Dubensky *et al., J. Virol.* 70:508 (1996)), Venezuelan equine encephalitis virus (see Johnston *et al.,* US 5,643,576) and rhabdoviruses, such as vesicular stomatitis virus (see Rose, WO 96/34625)and papillomaviruses (Ohe *et al., Human Gene Therapy* 6:325 (1995); Woo *et al.,* WO 94/12629 and Xiao & Brandsma, *Nucleic Acids. Res.* 24, 2630-2622 (1996)).

DNA encoding an immunogen, or a vector containing the same, can be packaged into liposomes. Suitable lipids and related analogs are described by Eppstein *et al.,* US 5,208,036, Felgner *et al.,* US 5,264,618, Rose, US 5,279,833, and Epand *et al.,* US 5,283,185. Vectors and DNA encoding an immunogen can also be adsorbed to or associated with particulate carriers, examples of which include polymethyl methacrylate polymers and polylactides and poly (lactide-co-glycolides), see, *e.g.,* McGee *et al., J. Micro Encap.* (1996).

Gene therapy vectors or naked polypeptides (*e.g.*, DNA) can be delivered *in vivo* by administration to an individual patient, typically by systemic administration (*e.g.*, intravenous, intraperitoneal, nasal, gastric, intradermal, intramuscular, subdermal, or intracranial infusion) or topical application (*see e.g.*, Anderson *et al.*, US 5,399,346).

5  The term "naked polynucleotide" refers to a polynucleotide not complexed with colloidal materials. Naked polynucleotides are sometimes cloned in a plasmid vector. Such vectors can further include facilitating agents such as bupivacine (Attardo *et al.*, US 5,593,970). DNA can also be administered using a gene gun. See Xiao & Brandsma, *supra*. The DNA encoding an immunogen is precipitated onto the surface of

10  microscopic metal beads. The microprojectiles are accelerated with a shock wave or expanding helium gas, and penetrate tissues to a depth of several cell layers. For example, The Accel™ Gene Delivery Device manufactured by Agacetus, Inc. Middleton WI is suitable. Alternatively, naked DNA can pass through skin into the blood stream simply by spotting the DNA onto skin with chemical or mechanical irritation (see

15  Howell *et al.*, WO 95/05853).

In a further variation, vectors encoding immunogens can be delivered to cells *ex vivo*, such as cells explanted from an individual patient (*e.g.*, lymphocytes, bone marrow aspirates, tissue biopsy) or universal donor hematopoietic stem cells, followed by reimplantation of the cells into a patient, usually after selection for cells which have

20  incorporated the vector.


## II. Prophylactic and Therapeutic Methods

The present invention is directed *inter alia* to treatment of Alzheimer's and other amyloidogenic diseases by administration of therapeutic immunological

25  reagents (*e.g.*, humanized immunoglobulins) to specific epitopes within Aβ to a patient under conditions that generate a beneficial therapeutic response in a patient (*e.g.*, induction of phagocytosis of Aβ, reduction of plaque burden, inhibition of plaque formation, reduction of neuritic dystrophy, improving cognitive function, and/or reversing, treating or preventing cognitive decline) in the patient, for example, for the

30  prevention or treatment of an amyloidogenic disease. The invention is also directed to use of the disclosed immunological reagents (*e.g.*, humanized immunoglobulins) in the

manufacture of a medicament for the treatment or prevention of an amyloidogenic disease.

The term "treatment" as used herein, is defined as the application or administration of a therapeutic agent to a patient, or application or administration of a
5    therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, a symptom of disease or a predisposition toward a disease, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease, the symptoms of disease or the predisposition toward disease.

In one aspect, the invention provides methods of preventing or treating a
10   disease associated with amyloid deposits of Aβ in the brain of a patient. Such diseases include Alzheimer's disease, Down's syndrome and cognitive impairment. The latter can occur with or without other characteristics of an amyloidogenic disease. Some methods of the invention entail administering an effective dosage of an antibody that specifically binds to a component of an amyloid deposit to the patient. Such methods
15   are particularly useful for preventing or treating Alzheimer's disease in human patients. Exemplary methods entail administering an effective dosage of an antibody that binds to Aβ. Preferred methods entail administering an effective dosage of an antibody that specifically binds to an epitope within residues 1-10 of Aβ, for example, antibodies that specifically bind to an epitope within residues 1-3 of Aβ, antibodies that specifically
20   bind to an epitope within residues 1-4 of Aβ, antibodies that specifically bind to an epitope within residues 1-5 of Aβ, antibodies that specifically bind to an epitope within residues 1-6 of Aβ, antibodies that specifically bind to an epitope within residues 1-7 of Aβ, or antibodies that specifically bind to an epitope within residues 3-7 of Aβ. In yet another aspect, the invention features administering antibodies that bind to an epitope
25   comprising a free N-terminal residue of Aβ. In yet another aspect, the invention features administering antibodies that bind to an epitope within residues of 1-10 of Aβ wherein residue 1 and/or residue 7 of Aβ is aspartic acid. In yet another aspect, the invention features administering antibodies that specifically bind to Aβ peptide without binding to full-length amyloid precursor protein (APP). In yet another aspect, the isotype of the
30   antibody is human IgG1.

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In yet another aspect, the invention features administering antibodies that bind to an amyloid deposit in the patient and induce a clearing response against the amyloid deposit. For example, such a clearing response can be effected by Fc receptor mediated phagocytosis.

5       Therapeutic agents of the invention are typically substantially pure from undesired contaminant. This means that an agent is typically at least about 50% w/w (weight/weight) purity, as well as being substantially free from interfering proteins and contaminants. Sometimes the agents are at least about 80% w/w and, more preferably at least 90 or about 95% w/w purity. However, using conventional protein purification

10      techniques, homogeneous peptides of at least 99% w/w can be obtained.

The methods can be used on both asymptomatic patients and those currently showing symptoms of disease. The antibodies used in such methods can be human, humanized, chimeric or nonhuman antibodies, or fragments thereof (*e.g.*, antigen binding fragments) and can be monoclonal or polyclonal, as described herein. In

15      yet another aspect, the invention features administering antibodies prepared from a human immunized with Aβ peptide, which human can be the patient to be treated with antibody.

In another aspect, the invention features administering an antibody with a pharmaceutical carrier as a pharmaceutical composition. Alternatively, the antibody can

20      be administered to a patient by administering a polynucleotide encoding at least one antibody chain. The polynucleotide is expressed to produce the antibody chain in the patient. Optionally, the polynucleotide encodes heavy and light chains of the antibody. The polynucleotide is expressed to produce the heavy and light chains in the patient. In exemplary embodiments, the patient is monitored for level of administered antibody in

25      the blood of the patient.

The invention thus fulfills a longstanding need for therapeutic regimes for preventing or ameliorating the neuropathology and, in some patients, the cognitive impairment associated with Alzheimer's disease.

A. Patients Amenable to Treatment

Patients amenable to treatment include individuals at risk of disease but not showing symptoms, as well as patients presently showing symptoms. In the case of Alzheimer's disease, virtually anyone is at risk of suffering from Alzheimer's disease if

5    he or she lives long enough. Therefore, the present methods 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 experienced this disease, and those whose risk is determined

10   by analysis of genetic or biochemical markers. Genetic markers of risk toward 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 (see Hardy, *supra*). Other markers of risk are mutations in the presenilin genes, PS1 and PS2, and ApoE4, family history of AD, hypercholesterolemia or

15   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 AD. These include measurement of CSF tau and A$\beta$42 levels. Elevated tau and decreased A$\beta$42 levels signify the presence of AD. Individuals suffering from

20   Alzheimer's disease can also be diagnosed by ADRDA criteria as discussed in the Examples section.

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

25   Treatment can be monitored by assaying antibody levels over time. If the response falls, a booster dosage is indicated. In the case of potential Down's syndrome patients, treatment can begin antenatally by administering therapeutic agent to the mother or shortly after birth.

B.  Treatment Regimes and Dosages

In prophylactic applications, pharmaceutical compositions or medicaments are administered to a patient susceptible to, or otherwise at risk of, Alzheimer's disease in an amount sufficient to eliminate or reduce the risk, lessen the severity, or delay 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 therapeutic applications, compositions or medicants are administered to a patient suspected of, or already suffering from such a disease 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 some methods, administration of agent reduces or eliminates myocognitive impairment in patients that have not yet developed characteristic Alzheimer's pathology.  An amount adequate to accomplish therapeutic or prophylactic treatment is defined as a therapeutically- or prophylactically-effective dose.  In both prophylactic and therapeutic regimes, agents are usually administered in several dosages until a sufficient immune response has been achieved.  The term "immune response" or "immunological response" includes the development of a humoral (antibody mediated) and/or a cellular (mediated by antigen-specific T cells or their secretion products) response directed against an antigen in a recipient subject.  Such a response can be an active response, i.e., induced by administration of immunogen, or a passive response, i.e., induced by administration of immunoglobulin or antibody or primed T-cells.

An "immunogenic agent" or "immunogen" is capable of inducing an immunological response against itself on administration to a mammal, optionally in conjunction with an adjuvant.  Typically, the immune response is monitored and repeated dosages are given if the immune response starts to wane.

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, target site, 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 non-human

mammals including transgenic mammals can also be treated. Treatment dosages need to be titrated to optimize safety and efficacy.

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For passive immunization with an antibody, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg, of the host body weight. For example dosages can be 1 mg/kg body weight or 10 mg/kg body weight or within the range of 1-10 mg/kg, preferably at least 1 mg/kg. Subjects can be administered such doses daily, on alternative days, weekly or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple dosages over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months. Exemplary dosage schedules include 1-10 mg/kg or 15 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated.

20

Antibody is usually administered on multiple occasions. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to Aβ in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of 1-1000 μg/ml and in some methods 25-300 μg/ml. Alternatively, antibody 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 antibodies show the longest half-life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies.

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The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, compositions containing the present antibodies or a cocktail thereof are administered to a patient not already in the disease state to enhance the patient's resistance. Such an amount is defined to be a "prophylactic effective dose." In this use, the precise amounts again depend upon the patient's state of health and general immunity, but generally range from 0.1 to 25 mg per dose, especially 0.5 to 2.5 mg per dose. 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 (*e.g.*, from about 1 to 200 mg of antibody per dose, with dosages of from 5 to 25 mg being more commonly

5    used) 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 patent can be administered a prophylactic regime.

Doses for nucleic acids encoding antibodies range from about 10 ng to

10   1 g, 100 ng to 100 mg, 1 µg to 10 mg, or 30-300 µg DNA per patient. Doses for infectious viral vectors vary from 10-100, or more, virions per dose.

Therapeutic agents can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intraperitoneal, intranasal or intramuscular means for prophylactic and/or therapeutic treatment. The most typical

15   route of administration of an immunogenic agent is subcutaneous although other routes can be equally effective. The next most common route is intramuscular injection. This type of injection is most typically 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. Intramuscular injection or intravenous

20   infusion are preferred for administration of antibody. In some methods, particular therapeutic antibodies are injected directly into the cranium. In some methods, antibodies are administered as a sustained release composition or device, such as a Medipad™ device.

Agents of the invention can optionally be administered in combination

25   with other agents that are at least partly effective in treatment of amyloidogenic disease. In the case of Alzheimer's and Down's syndrome, in which amyloid deposits occur in the brain, agents of the invention can also be administered in conjunction with other agents that increase passage of the agents of the invention across the blood-brain barrier.

## C. Pharmaceutical Compositions

Agents of the invention are often administered as pharmaceutical compositions comprising an active therapeutic agent, *i.e.*, and a variety of other pharmaceutically acceptable components. See *Remington's Pharmaceutical Science*

5     (15th ed., Mack Publishing Company, Easton, Pennsylvania (1980)). The preferred form depends on the intended mode of administration and therapeutic application. The compositions can also include, 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 administration. The

10    diluent is 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. In addition, the pharmaceutical composition or formulation may also include other carriers, adjuvants, or nontoxic, nontherapeutic, nonimmunogenic stabilizers and the like.

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

20    function as immunostimulating agents (*i.e.*, adjuvants).

For parenteral administration, agents of the invention can be administered as injectable dosages of a solution or suspension of the substance in a physiologically acceptable diluent with a pharmaceutical carrier that can be a sterile liquid such as water oils, saline, glycerol, or ethanol. Additionally, auxiliary substances, such as wetting or

25    emulsifying agents, surfactants, pH buffering substances and the like 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 preferred liquid carriers, particularly for injectable solutions. Antibodies can be

30    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. An exemplary composition comprises monoclonal antibody at 5 mg/mL, formulated in

aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCl.

Typically, compositions are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid

5    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 copolymer for enhanced adjuvant effect, as discussed above (see Langer, *Science* 249: 1527 (1990) and Hanes, *Advanced Drug Delivery Reviews* 28:97 (1997)). The agents of this invention can be administered in the form of a depot injection or implant

10   preparation, which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

Additional formulations suitable for other modes of administration include oral, intranasal, and pulmonary formulations, suppositories, and transdermal applications. For suppositories, binders and carriers include, for example, polyalkylene

15   glycols or triglycerides; such suppositories can be formed from mixtures containing the active ingredient in the range of 0.5% to 10%, preferably 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

20   release formulations or powders and contain 10%-95% of active ingredient, preferably 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

25   Glenn *et al.*, *Nature* 391, 851 (1998)). 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.*, *Eur. J. Immunol.* 25:3521 (1995); Cevc *et al.*,

30   *Biochem. Biophys. Acta* 1368:201-15 (1998)).

III.  Monitoring the Course of Treatment

The invention provides methods of monitoring treatment in a patient suffering from or susceptible to Alzheimer's, *i.e.*, for monitoring a course of treatment being administered to a patient.  The methods can be used to monitor both therapeutic treatment on symptomatic patients and prophylactic treatment on asymptomatic patients.  In particular, the methods are useful for monitoring passive immunization (*e.g.*, measuring level of administered antibody).

Some methods entail determining a baseline value, for example, of an antibody level or profile in a patient, before administering a dosage of agent, and comparing this with a value for the profile or level after treatment.  A significant increase (*i.e.*, greater than the typical margin of experimental error in repeat measurements of the same sample, expressed as one standard deviation from the mean of such measurements) in value of the level or profile signals a positive treatment outcome (*i.e.*, that administration of the agent has achieved a desired response).  If the value for immune response does not change significantly, or decreases, a negative treatment outcome is indicated.

In other methods, a control value (*i.e.*, a mean and standard deviation) of level or profile is determined for a control population.  Typically the individuals in the control population have not received prior treatment.  Measured values of the level or profile in a patient after administering a therapeutic agent are then compared with the control value.  A significant increase relative to the control value (*e.g.*, greater than one standard deviation from the mean) signals a positive or sufficient treatment outcome.  A lack of significant increase or a decrease signals a negative or insufficient treatment outcome.  Administration of agent is generally continued while the level is increasing relative to the control value.  As before, attainment of a plateau relative to control values is an indicator that the administration of treatment can be discontinued or reduced in dosage and/or frequency.

In other methods, a control value of the level or profile (*e.g.*, a mean and standard deviation) is determined from a control population of individuals who have undergone treatment with a therapeutic agent and whose levels or profiles have plateaued in response to treatment.  Measured values of levels or profiles in a patient are compared with the control value.  If the measured level in a patient is not significantly

different (*e.g.*, more than one standard deviation) from the control value, treatment can be discontinued. If the level in a patient is significantly below the control value, continued administration of agent is warranted. If the level in the patient persists below the control value, then a change in treatment may be indicated.

5          In other methods, a patient who is not presently receiving treatment but has undergone a previous course of treatment is monitored for antibody levels or profiles to determine whether a resumption of treatment is required. The measured level or profile in the patient can be compared with a value previously achieved in the patient after a previous course of treatment. A significant decrease relative to the previous

10    measurement (*i.e.*, greater than a typical margin of error in repeat measurements of the same sample) is an indication that treatment can be resumed. Alternatively, the value measured in a patient can be compared with a control value (mean plus standard deviation) determined in a population of patients after undergoing a course of treatment. Alternatively, the measured value in a patient can be compared with a control value in

15    populations of prophylactically treated patients who remain free of symptoms of disease, or populations of therapeutically treated patients who show amelioration of disease characteristics. In all of these cases, a significant decrease relative to the control level (*i.e.*, more than a standard deviation) is an indicator that treatment should be resumed in a patient.

20          The tissue sample for analysis is typically blood, plasma, serum, mucous fluid or cerebrospinal fluid from the patient. The sample is analyzed, for example, for levels or profiles of antibodies to Aβ peptide, *e.g.*, levels or profiles of humanized antibodies. ELISA methods of detecting antibodies specific to Aβ are described in the Examples section. In some methods, the level or profile of an administered antibody is

25    determined using a clearing assay, for example, in an *in vitro* phagocytosis assay, as described herein. In such methods, a tissue sample from a patient being tested is contacted with amyloid deposits (*e.g.*, from a PDAPP mouse) and phagocytic cells bearing Fc receptors. Subsequent clearing of the amyloid deposit is then monitored. The existence and extent of clearing response provides an indication of the existence and

30    level of antibodies effective to clear Aβ in the tissue sample of the patient under test.

The antibody profile following passive immunization typically shows an immediate peak in antibody concentration followed by an exponential decay. Without a further dosage, the decay approaches pretreatment levels within a period of days to months depending on the half-life of the antibody administered. For example the half-

5    life of some human antibodies is of the order of 20 days.

In some methods, a baseline measurement of antibody to Aβ in the patient is made before administration, a second measurement is made soon thereafter to determine the peak antibody level, and one or more further measurements are made at intervals to monitor decay of antibody levels. When the level of antibody has declined

10   to baseline or a predetermined percentage of the peak less baseline (e.g., 50%, 25% or 10%), administration of a further dosage of antibody is administered. In some methods, peak or subsequent measured levels less background are compared with reference levels previously determined to constitute a beneficial prophylactic or therapeutic treatment regime in other patients. If the measured antibody level is significantly less than a

15   reference level (e.g., less than the mean minus one standard deviation of the reference value in population of patients benefiting from treatment) administration of an additional dosage of antibody is indicated.

Additional methods include monitoring, over the course of treatment, any art-recognized physiologic symptom (e.g., physical or mental symptom) routinely relied

20   on by researchers or physicians to diagnose or monitor amyloidogenic diseases (e.g., Alzheimer's disease). For example, one can monitor cognitive impairment. The latter is a symptom of Alzheimer's disease and Down's syndrome but can also occur without other characteristics of either of these diseases. For example, cognitive impairment can be monitored by determining a patient's score on the Mini-Mental State Exam in

25   accordance with convention throughout the course of treatment.


C. Kits

The invention further provides kits for performing the monitoring methods described above. Typically, such kits contain an agent that specifically binds to

30   antibodies to Aβ. The kit can also include a label. For detection of antibodies to Aβ, the label is typically in the form of labeled anti-idiotypic antibodies. For detection of antibodies, the agent can be supplied prebound to a solid phase, such as to the wells of a

microtiter dish. Kits also typically contain labeling providing directions for use of the kit. The labeling may also include a chart or other correspondence regime correlating levels of measured label with levels of antibodies to Aβ. The term labeling refers to any written or recorded material that is attached to, or otherwise accompanies a kit at any

5    time during its manufacture, transport, sale or use. For example, the term labeling encompasses advertising leaflets and brochures, packaging materials, instructions, audio or videocassettes, computer discs, as well as writing imprinted directly on kits.

The invention also provides diagnostic kits, for example, research, detection and/or diagnostic kits (e.g., for performing in vivo imaging). Such kits

10   typically contain an antibody for binding to an epitope of Aβ, preferably within residues 1-10. Preferably, the antibody is labeled or a secondary labeling reagent is included in the kit. Preferably, the kit is labeled with instructions for performing the intended application, for example, for performing an in vivo imaging assay. Exemplary antibodies are those described herein.

15

### D. In vivo Imaging

The invention provides methods of in vivo imaging amyloid deposits in a patient. Such methods are useful to diagnose or confirm diagnosis of Alzheimer's disease, or susceptibility thereto. For example, the methods can be used on a patient

20   presenting with symptoms of dementia. If the patient has abnormal amyloid deposits, then the patient is likely suffering from Alzheimer's disease. The methods can also be used on asymptomatic patients. Presence of abnormal deposits of amyloid indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in patients who have been previously

25   diagnosed with Alzheimer's disease.

The methods work by administering a reagent, such as antibody that binds to Aβ, to the patient and then detecting the agent after it has bound. Preferred antibodies bind to Aβ deposits in a patient without binding to full length APP polypeptide. Antibodies binding to an epitope of Aβ within amino acids 1-10 are particularly

30   preferred. In some methods, the antibody binds to an epitope within amino acids 7-10 of Aβ. Such antibodies typically bind without inducing a substantial clearing response. In other methods, the antibody binds to an epitope within amino acids 1-7 of Aβ. Such

antibodies typically bind and induce a clearing response to Aβ. However, the clearing response can be avoided by using antibody fragments lacking a full-length constant region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent. In general, antibodies binding to epitopes C-terminal to residue

5        10 of Aβ do not show as strong a signal as antibodies binding to epitopes within residues 1-10, presumably because the C-terminal epitopes are inaccessible in amyloid deposits. Accordingly, such antibodies are less preferred.

         Diagnostic reagents can be administered by intravenous injection into the body of the patient, or directly into the brain by intracranial injection or by drilling a

10       hole through the skull. The dosage of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for Aβ is unlabelled and a secondary labeling agent is used to bind to the primary reagent. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical detection. Use of paramagnetic

15       labels is suitable for tomographic detection without surgical intervention. Radioactive labels can also be detected using PET or SPECT.

         Diagnosis is performed by comparing the number, size, and/or intensity of labeled loci, to corresponding baseline values. The base line values can represent the mean levels in a population of undiseased individuals. Baseline values can also

20       represent previous levels determined in the same patient. For example, baseline values can be determined in a patient before beginning treatment, and measured values thereafter compared with the baseline values. A decrease in values relative to baseline signals a positive response to treatment.


25       The present invention will be more fully described by the following non-limiting examples.

# EXAMPLES

## Example I.: Therapeutic Efficacy of Anti-Aβ Antibodies:  mAb 2H3, mAb 10D5, mAb 266, mAb 21F12 and pAb Aβ1-42

5        This example tests the capacity of various monoclonal and polyclonal antibodies to Aβ to inhibit accumulation of Aβ in the brain of heterozygotic transgenic mice.

### A.  Study Design

10       Sixty male and female, heterozygous PDAPP transgenic mice, 8.5 to 10.5 months of age were obtained from Charles River Laboratory.  The mice were sorted into six groups to be treated with various antibodies directed to Aβ.  Animals were distributed to match the gender, age, parentage and source of the animals within the groups as closely as possible.  Table 2 depicts the Experimental design.

15

Table 2:  Experimental Design

| Treatment Group | $N^a$ | Treatment Antibody | Antibody Specificity | Antibody Isotype |
|---|---|---|---|---|
| 1 | 9 | none (PBS alone) | $NA^b$ | NA |
| 2 | 10 | Polyclonal | Aβ1-42 | mixed |
| 3 | 0 | mAb$^d$ 2H3 | Aβ1-12 | IgG1 |
| 4 | 8 | mAb 10D5 | Aβ3-7 | IgG1 |
| 5 | 6 | mAb 266 | Aβ13-28 | IgG1 |
| 6 | 8 | mAb 21F12 | Aβ33-42 | IgG2a |

a.  Number of mice in group at termination of the experiment.  All groups started with 10 animals per group.
b.  NA: not applicable
c.  mouse polyclonal: anti-aggregated Aβ42
20    d.  mAb: monoclonal antibody

As shown in Table 2, the antibodies included four murine Aβ-specific monoclonal antibodies, 2H3 (directed to Aβ residues 1-12), 10D5 (directed to Aβ residues 3-7), 266 (directed to Aβ residues 13-28 and binds to soluble but not to aggregated AN1792), 21F12 (directed to Aβ residues 33-42). A fifth group was treated

5   with an Aβ-specific polyclonal antibody fraction (raised by immunization with aggregated AN1792). The negative control group received the diluent, PBS, alone without antibody.


### B. Monitoring the Course of Treatment

10   The monoclonal antibodies were injected at a dose of about 10 mg/kg (assuming that the mice weighed 50 g). Antibody titers were monitored over the 28 weeks of treatment. Injections were administered intraperitoneally every seven days on average to maintain anti-Aβ titers above 1000. Although lower titers were measured for mAb 266 since it does not bind well to the aggregated AN1792 used as the capture

15   antigen in the assay, the same dosing schedule was maintained for this group. The group receiving monoclonal antibody 2H3 was discontinued within the first three weeks since the antibody was cleared too rapidly *in vivo*.

For determination of antibody titers, a subset of three randomly chosen mice from each group were bled just prior to each intraperitoneal inoculation, for a total

20   of 30 bleeds. Antibody titers were measured as Aβ1-42-binding antibody using a sandwich ELISA with plastic multi-well plates coated with Aβ1-42 as described in detail in the General Materials and Methods. Mean titers for each bleed are set forth in Table 3 for the polyclonal antibody and the monoclonals 10D5 and 21F12.

Table 3:

| weeks 21F12 | 21F12 | weeks 10D5 | 10D5 | weeks poly | poly |
|---|---|---|---|---|---|
| 0.15 | 500 | 0.15 | 3000 | 0.15 | 1600 |
| 0.5 | 800 | 0.5 | 14000 | 0.5 | 4000 |
| 1 | 2500 | 1 | 5000 | 1 | 4500 |
| 1.5 | 1800 | 1.1 | 5000 | 1.5 | 3000 |
| 2 | 1400 | 1.2 | 1300 | 2 | 1300 |
| 3 | 6000 | 2 | 3000 | 3 | 1600 |
| 3.5 | 550 | 3 | 4000 | 3.5 | 650 |
| 4 | 1600 | 3.5 | 500 | 4 | 1300 |
| 5 | 925 | 4 | 2400 | 5 | 450 |
| 6 | 3300 | 5 | 925 | 6 | 2100 |
| 7 | 4000 | 6 | 1700 | 7 | 1300 |
| 8 | 1400 | 7 | 1600 | 8 | 2300 |

| | | | | | |
|---|---|---|---|---|---|
| 9 | 1900 | 8 | 4000 | 9 | 700 |
| 10 | 1700 | 9 | 1800 | 10 | 600 |
| 11 | 1600 | 10 | 1800 | 11 | 600 |
| 12 | 1000 | 11 | 2300 | 12 | 1000 |
| 13 | 1500 | 12 | 2100 | 13 | 900 |
| 14 | 1300 | 13 | 2800 | 14 | 1900 |
| 15 | 1000 | 14 | 1900 | 15 | 1200 |
| 16 | 1700 | 15 | 2700 | 16 | 700 |
| 17 | 1700 | 16 | 1300 | 17 | 2100 |
| 18 | 5000 | 17 | 2200 | 18 | 1800 |
| 19 | 900 | 18 | 2200 | 19 | 1800 |
| 20 | 300 | 19 | 2500 | 20 | 1200 |
| 22 | 1750 | 20 | 980 | 22 | 1000 |
| 23 | 1600 | 22 | 2000 | 23 | 1200 |
| 24 | 1000 | 23 | 1000 | 24 | 675 |
| 25 | 1100 | 24 | 850 | 25 | 850 |
| 26 | 2250 | 25 | 600 | 26 | 1600 |
| 27 | 1400 | 26 | 1100 | 27 | 1900 |
| 28 | | 27 | 1450 | 28 | |
| | | 28 | | | |

Titers averaged about 1000 over this time period for the polyclonal antibody preparation and were slightly above this level for the 10D5- and 21F12-treated animals.

5          Treatment was continued over a six-month period for a total of 196 days. Animals were euthanized one week after the final dose.


### C. Aβ and APP Levels in the Brain:

10          Following about six months of treatment with the various anti-Aβ antibody preparations, brains were removed from the animals following saline perfusion. One hemisphere was prepared for immunohistochemical analysis and the second was used for the quantitation of Aβ and APP levels. To measure the concentrations of various forms of beta amyloid peptide and amyloid precursor protein (APP), the

15     hemisphere was dissected and homogenates of the hippocampal, cortical, and cerebellar regions were prepared in 5M guanidine. These were serially diluted and the level of amyloid peptide or APP was quantitated by comparison to a series of dilutions of standards of Aβ peptide or APP of known concentrations in an ELISA format.

The levels of total Aβ and of Aβ1-42 measured by ELISA in

20     homogenates of the cortex, and the hippocampus and the level of total Aβ in the cerebellum are shown in Tables 4, 5, and 6, respectively. The median concentration of

total Aβ for the control group, inoculated with PBS, was 3.6-fold higher in the

hippocampus than in the cortex (median of 63,389 ng/g hippocampal tissue compared to

17,818 ng/g for the cortex). The median level in the cerebellum of the control group

(30.6 ng/g tissue) was more than 2,000-fold lower than in the hippocampus. These

5    levels are similar to those previously reported for heterozygous PDAPP transgenic mice

of this age (Johnson-Wood *et al.*, *supra*).

        For the cortex, one treatment group had a median Aβ level, measured as

Aβ1-42, which differed significantly from that of the control group (p < 0.05), those

animals receiving the polyclonal anti-Aβ antibody as shown in Table 4. The median

10   level of Aβ1-42 was reduced by 65%, compared to the control for this treatment group.

The median levels of Aβ1-42 were also significantly reduced by 55% compared to the

control in one additional treatment group, those animals dosed with the mAb 10D5 (p =

0.0433).

ELN-002PC

Table 4

### CORTEX

| Treatment Group | N[a] | Medians | | | | | | Means | |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| | | Total Aβ | | | Aβ42 | | | Total Aβ | Aβ42 |
| | | ELISA value[b] | P value[c] | % Change | ELISA value | P value | % Change | ELISA value | ELISA value |
| PBS | 9 | 17818 | NA[d] | NA | 13802 | NA | NA | 16150+/-7456[e] | 12621+/-5738 |
| Polyclonal anti-Aβ42 | 10 | 6160 | 0.0055 | -65 | 4892 | 0.0071 | -65 | 5912+/-4492 | 4454+/-3347 |
| mAb 10D5 | 8 | 7915 | 0.1019 | -56 | 6214 | 0.0433 | -55 | 9695+/-6929 | 6943+/-3351 |
| mAb 266 | 6 | 9144 | 0.1255 | -49 | 8481 | 0.1255 | -39 | 9204+/-9293 | 7489+/-6921 |
| mAb 21F12 | 8 | 15158 | 0.2898 | -15 | 13578 | 0.7003 | -2 | 12481+/-7082 | 11005+/-6324 |

Footnotes:
a. Number of animals per group at the end of the experiment
b. ng/g tissue
c. Mann Whitney analysis
d. NA: not applicable
e. Standard Deviation

5

In the hippocampus, the median percent reduction of total Aβ associated with treatment with polyclonal anti-Aβ antibody (50%, p = 0.0055) was not as great as that observed in the cortex (65%) (Table 5). However, the absolute magnitude of the reduction was almost 3-fold greater in the hippocampus than in the cortex, a net reduction of 31,683 ng/g tissue in the hippocampus versus 11,658 ng/g tissue in the cortex. When measured as the level of the more amyloidogenic form of Aβ, Aβ1-42, rather than as total Aβ, the reduction achieved with the polyclonal antibody was significant (p = 0.0025). The median levels in groups treated with the mAbs 10D5 and 266 were reduced by 33% and 21%, respectively.

ELN-002PC

Table 5

<u>HIPPOCAMPUS</u>

| Treatment Group | N[a] | Medians | | | | | | Means | |
|---|---|---|---|---|---|---|---|---|---|
| | | Total Aβ | | | Aβ42 | | | Total Aβ | Aβ42 |
| | | ELISA value[b] | P value[c] | % Change | ELISA value | P value | % Change | ELISA value | ELISA value |
| PBS | 9 | 63389 | NA[d] | NA | 54429 | NA | NA | 58351+/-13308[e] | 52801+/-14701 |
| Polyclonal anti-Aβ42 | 10 | 31706 | 0.0055 | -50 | 27127 | 0.0025 | -50 | 30058+/-22454 | 24853+/-18262 |
| mAb 10D5 | 8 | 46779 | 0.0675 | -26 | 36290 | 0.0543 | -33 | 44581+/-18632 | 36465+/-17146 |
| mAb 266 | 6 | 48689 | 0.0990 | -23 | 43034 | 0.0990 | -21 | 36419+/-27304 | 32919+/-25372 |
| mAb 21F12 | 8 | 51563 | 0.7728 | -19 | 47961 | 0.8099 | -12 | 57327+/-28927 | 50305+/-23927 |

a. Number of animals per group at the end of the experiment
b. ng/g tissue
c. Mann Whitney analysis
d. NA: not applicable
e. Standard Deviation

5

Total Aβ was also measured in the cerebellum (Table 6). Those groups dosed with the polyclonal anti-Aβ and the 266 antibody showed significant reductions of the levels of total Aβ (43% and 46%, p = 0.0033 and p = 0.0184, respectively) and that

5   group treated with 10D5 had a near significant reduction (29%, p = 0.0675).

Table 6

| CEREBELLUM | | | | | |
|---|---|---|---|---|---|
| Treatment Group | $N^a$ | Medians | | | Means |
| | | Total Aβ | | | Total Aβ |
| | | ELISA value[b] | P value[c] | % Change | ELISA value |
| PBS | 9 | 30.64 | NA[d] | NA | 40.00+/-31.89[e] |
| Polyclonal anti-Aβ42 | 10 | 17.61 | 0.0033 | -43 | 18.15+/-4.36 |
| mAb 10D5 | 8 | 21.68 | 0.0675 | -29 | 27.29+/-19.43 |
| mAb 266 | 6 | 16.59 | 0.0184 | -46 | 19.59+/-6.59 |
| mAb 21F12 | 8 | 29.80 | >0.9999 | -3 | 32.88+/-9.90 |

a. Number of animals per group at the end of the experiment
b. ng/g tissue
10  c. Mann Whitney analysis
d. NA: not applicable
e. Standard Deviation

APP concentration was also determined by ELISA in the cortex and

15  cerebellum from antibody-treated and control, PBS-treated mice. Two different APP assays were utilized. The first, designated APP-α/FL, recognizes both APP-alpha (α, the secreted form of APP which has been cleaved within the Aβ sequence), and full-length forms (FL) of APP, while the second recognizes only APP-α. In contrast to the treatment-associated diminution of Aβ in a subset of treatment groups, the levels of APP

were virtually unchanged in all of the treated compared to the control animals. These results indicate that the immunizations with Aβ antibodies deplete Aβ without depleting APP.

In summary, Aβ levels were significantly reduced in the cortex, hippocampus and cerebellum in animals treated with the polyclonal antibody raised against AN1792. To a lesser extent monoclonal antibodies to the amino terminal region of Aβ1-42, specifically amino acids 1-16 and 13-28 also showed significant treatment effects.

## D. Histochemical Analyses:

The morphology of Aβ-immunoreactive plaques in subsets of brains from mice in the PBS, polyclonal Aβ42, 21F12, 266 and 10D5 treatment groups was qualitatively compared to that of previous studies in which standard immunization procedures with Aβ42 were followed.

The largest alteration in both the extent and appearance of amyloid plaques occurred in the animals immunized with the polyclonal Aβ42 antibody. The reduction of amyloid load, eroded plaque morphology and cell-associated Aβ immunoreactivity closely resembled effects produced by the standard immunization procedure. These observations support the ELISA results in which significant reductions in both total Aβ and Aβ42 were achieved by administration of the polyclonal Aβ42 antibody.

In similar qualitative evaluations, amyloid plaques in the 10D5 group were also reduced in number and appearance, with some evidence of cell-associated Aβ immunoreactivity. Relative to control-treated animals, the polyclonal Ig fraction against Aβ and one of the monoclonal antibodies (10D5) reduced plaque burden by 93% and 81%, respectively ($p < 0.005$). 21F12 appeared to have a relatively modest effect on plaque burden. Micrographs of brain after treatment with pAbAβ$_{1-42}$ show diffuse deposits and absence of many of the larger compacted plaques in the pAbAβ$_{1-42}$ treated group relative to control treated animals.

E. Lymphoproliferative Responses

    Aβ-dependent lymphoproliferation was measured using spleen cells harvested eight days following the final antibody infusion. Freshly harvested cells, $10^5$

5   per well, were cultured for 5 days in the presence of Aβ1-40 at a concentration of 5 μM for stimulation. As a positive control, additional cells were cultured with the T cell mitogen, PHA, and, as a negative control, cells were cultured without added peptide.

    Splenocytes from aged PDAPP mice passively immunized with various anti-Aβ antibodies were stimulated *in vitro* with AN1792 and proliferative and cytokine

10  responses were measured. The purpose of these assays was to determine if passive immunization facilitated antigen presentation, and thus priming of T cell responses specific for AN1792. No AN1792-specific proliferative or cytokine responses were observed in mice passively immunized with the anti-Aβ antibodies.

15  **Example II: Therapeutic Efficacy of Anti-Aβ Antibodies: mAb 2H3, mAb 10D5,**
            **mAb 266, mAb 21F12, mAb 3D6, mAb 16C11 and pAb Aβ1-42**

    In a second study, treatment with 10D5 was repeated and two additional anti-Aβ antibodies were tested, monoclonals 3D6 (Aβ1-5) and 16C11 (Aβ33-42). Control groups received either PBS or an irrelevant isotype-matched antibody (TM2a).

20  The mice were older (11.5-12 month old heterozygotes) than in the previous study, otherwise the experimental design was the same. Once again, after six months of treatment, 10D5 reduced plaque burden by greater than 80% relative to either the PBS or isotype-matched antibody controls (p=0.003). One of the other antibodies against Aβ, 3D6, was equally effective, producing an 86% reduction (p=0.003). In contrast, the

25  third antibody against the peptide, 16C11, failed to have any effect on plaque burden. Similar findings were obtained with Aβ42 ELISA measurements.

    These results demonstrate that an antibody response against Aβ peptide, in the absence of T cell immunity, is sufficient to decrease amyloid deposition in PDAPP mice, but that not all anti-Aβ antibodies are equally efficacious. Antibodies directed to

30  epitopes comprising amino acids 1-5 or 3-7 of Aβ are particularly efficacious. In summary, it can be demonstrated that passively administered antibodies against Aβ (*i.e.,*

passive immunization) reduces the extent of plaque deposition in a mouse model of Alzheimer's disease.

### Example III:  Monitoring of Antibody Binding in the CNS

5         This Example demonstrates that when held at modest serum concentrations (25–70 µg/ml), the antibodies gained access to the CNS at levels sufficient to decorate β-amyloid plaques.

To determine whether antibodies against Aβ could be acting directly within the CNS, brains taken from saline-perfused mice at the end of the Example II,

10       were examined for the presence of the peripherally-administered antibodies. Unfixed cryostat brain sections were exposed to a fluorescent reagent against mouse immunoglobulin (goat anti-mouse IgG-Cy3).  Plaques within brains of the 10D5 and 3D6 groups were strongly decorated with antibody, while there was no staining in the 16C11 group.  To reveal the full extent of plaque deposition, serial sections of each brain

15       were first immunoreacted with an anti-Aβ antibody, and then with the secondary reagent. 10D5 and 3D6, following peripheral administration,  gained access to most plaques within the CNS.  The plaque burden was greatly reduced in these treatment groups compared to the 16C11 group.  Antibody entry into the CNS was not due to abnormal leakage of the blood-brain barrier since there was no increase in vascular

20       permeability as measured by Evans Blue in PDAPP mice.  In addition, the concentration of antibody in the brain parenchyma of aged PDAPP mice was the same as in non-transgenic mice, representing 0.1% of the antibody concentration in serum (regardless of isotype).

These data indicate that peripherally administered antibodies can enter

25       the CNS where they can directly trigger amyloid clearance.  It is likely that 16C11 also had access to the plaques but was unable to bind.

### Example IV: *Ex vivo* Screening Assay for Activity of an Antibody Against Amyloid Deposits

30       To examine the effect of antibodies on plaque clearance, we established an *ex vivo* assay in which primary microglial cells were cultured with unfixed cryostat sections of either PDAPP mouse or human AD brains.  Microglial cells were obtained

from the cerebral cortices of neonate DBA/2N mice (1-3 days). The cortices were

mechanically dissociated in HBSS⁻ (Hanks' Balanced Salt Solution, Sigma) with 50

μg/ml DNase I (Sigma). The dissociated cells were filtered with a 100 μm cell strainer

(Falcon), and centrifuged at 1000 rpm for 5 minutes. The pellet was resuspended in

5       growth medium (high glucose DMEM, 10%FBS, 25ng/ml rmGM-CSF), and the cells

were plated at a density of 2 brains per T-75 plastic culture flask. After 7-9 days, the

flasks were rotated on an orbital shaker at 200 rpm for 2h at 37°C. The cell suspension

was centrifuged at 1000rpm and resuspended in the assay medium.

            10-μm cryostat sections of PDAPP mouse or human AD brains (post-

10      mortem interval < 3hr) were thaw mounted onto poly-lysine coated round glass

coverslips and placed in wells of 24-well tissue culture plates. The coverslips were

washed twice with assay medium consisting of H-SFM (Hybridoma-serum free medium,

Gibco BRL) with 1% FBS, glutamine, penicillin/streptomycin, and 5ng/ml rmGM-CSF

(R&D). Control or anti-Aβ antibodies were added at a 2x concentration (5 μg/ml final)

15      for 1 hour. The microglial cells were then seeded at a density of $0.8x\ 10^6$ cells/ml assay

medium. The cultures were maintained in a humidified incubator (37°C, 5%CO₂) for

24hr or more. At the end of the incubation, the cultures were fixed with 4%

paraformaldehyde and permeabilized with 0.1% Triton-X100. The sections were stained

with biotinylated 3D6 followed by a streptavidin / Cy3 conjugate (Jackson

20      ImmunoResearch). The exogenous microglial cells were visualized by a nuclear stain

(DAPI). The cultures were observed with an inverted fluorescent microscope (Nikon,

TE300) and photomicrographs were taken with a SPOT digital camera using SPOT

software (Diagnostic instruments). For Western blot analysis, the cultures were

extracted in 8M urea, diluted 1:1 in reducing tricine sample buffer and loaded onto a

25      16% tricine gel (Novex). After transfer onto immobilon, blots were exposed to 5 μg/ml

of the pabAβ42 followed by an HRP-conjugated anti-mouse antibody, and developed

with ECL (Amersham)

            When the assay was performed with PDAPP brain sections in the

presence of 16C11 (one of the antibodies against Aβ that was not efficacious *in vivo*), β-

30      amyloid plaques remained intact and no phagocytosis was observed. In contrast, when

adjacent sections were cultured in the presence of 10D5, the amyloid deposits were

largely gone and the microglial cells showed numerous phagocytic vesicles containing

74

Aβ. Identical results were obtained with AD brain sections; 10D5 induced phagocytosis of AD plaques, while 16C11 was ineffective. In addition, the assay provided comparable results when performed with either mouse or human microglial cells, and with mouse, rabbit, or primate antibodies against Aβ.

Table 7 compares Aβ binding versus phagocytosis for several different antibody binding specificities. It can be seen that antibodies binding to epitopes within aa 1-7 both bind and clear amyloid deposits, whereas antibodies binding to epitopes within amino acids 4-10 bind without clearing amyloid deposits. Antibodies binding to epitopes C-terminal to residue 10 neither bind nor clear amyloid deposits.

Table 7:  Analysis of Epitope Specificity

| | **Antibody** epitope | isotype | Staining | Phagocytosis |
|---|---|---|---|---|
| **N-Term** | | | | |
| mab | | | | |
| 3D6 | 1-5 | IgG2b | + | + |
| 10D5 | 3-7 | IgG1 | + | + |
| 22C8 | 3-7 | IgG2a | + | + |
| 6E10 | 5-10 | IgG1 | + | - |
| 14A8 | 4-10 | rat IgG1 | + | - |
| | | | | |
| **aa 13-28** | | | | |
| 18G11 | 10-18 | rat IgG1 | - | - |
| 266 | 16-24 | IgG1 | - | - |
| 22D12 | 18-21 | IgG2b | - | - |
| | | | | |
| **C-Term** | | | | |
| 2G3 | -40 | IgG1 | - | - |
| 16C11 | -40/-42 | IgG1 | - | - |
| 21F12 | -42 | IgG2a | - | - |
| | | | | |
| **Immune serum** | | | | |
| rabbit (CFA) | 1-6 | | + | + |
| mouse (CFA) | 3-7 | | + | + |
| mouse (QS-21) | 3-7 | | + | + |
| monkey (QS-21) | 1-5 | | + | + |
| mouse (MAP1-7) | | | + | + |

Table 8 shows results obtained with several antibodies against Aβ, comparing their abilities to induce phagocytosis in the *ex vivo* assay and to reduce *in vivo* plaque burden in passive transfer studies. Although 16C11 and 21F12 bound to aggregated

5      synthetic Aβ peptide with high avidity, these antibodies were unable to react with β-amyloid plaques in unfixed brain sections, could not trigger phagocytosis in the *ex vivo* assay, and were not efficacious *in vivo*. 10D5, 3D6, and the polyclonal antibody against Aβ were active by all three measures. These results show that efficacy *in vivo* is due to direct antibody mediated clearance of the plaques within the CNS, and that the *ex vivo*

10     assay is predictive of *in vivo* efficacy.


Table 8:   The *ex vivo* assay as predictor of *in vivo* efficacy

| Antibody | Isotype | Avidity for aggregated Aβ (pM) | Binding to β-amyloid plaques | *Ex vivo* efficacy | *In vivo* efficacy |
|---|---|---|---|---|---|
| **monoclonal** | | | | | |
| 3D6 | IgG2b | 470 | + | + | + |
| 10D5 | IgG1 | 43 | + | + | + |
| 16C11 | IgG1 | 90 | - | - | - |
| 21F12 | IgG2a | 500 | - | - | - |
| TM2a | IgG1 | - | - | - | - |
| **polyclonal** | | | | | |
| 1-42 | mix | 600 | + | + | + |


The same assay has been used to test clearing activity of an antibody

15     against a fragment of synuclein referred to as NAC. Synuclein has been shown to be an amyloid plaque-associated protein. An antibody to NAC was contacted with a brain tissue sample containing amyloid plaques, and microglial cells, as before. Rabbit serum was used as a control. Subsequent monitoring showed a marked reduction in the number and size of plaques indicative of clearing activity of the antibody.

20     Confocal microscopy was used to confirm that Aβ was internalized during the course of the *ex vivo* assay. In the presence of control antibodies, the exogenous microglial cells remained in a confocal plane above the tissue, there were no

phagocytic vesicles containing Aβ, and the plaques remained intact within the section. In the presence of 10D5, nearly all plaque material was contained in vesicles within the exogenous microglial cells. To determine the fate of the internalized peptide, 10D5 treated cultures were extracted with 8M urea at various time-points, and examined by

5      Western blot analysis. At the one hour time point, when no phagocytosis had yet occurred, reaction with a polyclonal antibody against Aβ revealed a strong 4 kD band (corresponding to the Aβ peptide). Aβ immunoreactivity decreased at day 1 and was absent by day 3. Thus, antibody-mediated phagocytosis of Aβ leads to its degradation.

To determine if phagocytosis in the *ex vivo* assay was Fc-mediated,

10     F(ab')2 fragments of the anti-Aß antibody 3D6 were prepared. Although the F(ab')2 fragments retained their full ability to react with plaques, they were unable to trigger phagocytosis by microglial cells. In addition, phagocytosis with the whole antibody could be blocked by a reagent against murine Fc receptors (anti-CD16/32). These data indicate that *in vivo* clearance of Aβ occurs through Fc-receptor mediated phagocytosis.

15

### Example V:    Passage of Antibodies Through the Blood-Brain Barrier

This example determines the concentration of antibody delivered to the brain following intravenous injection into a peripheral tissue of either normal or PDAPP mice. Following treatment, PDAPP or control normal mice were perfused with 0.9%

20     NaCl. Brain regions (hippocampus or cortex) were dissected and rapidly frozen. Brain were homogenized in 0.1% triton + protease inhibitors. Immunoglobulin was detected in the extracts by ELISA. F(ab)'2 goat anti-mouse IgG were coated onto an RIA plate as capture reagent. The serum or the brain extracts were incubated for 1hr. The isotypes were detected with anti-mouse IgG1-HRP or IgG2a-HRP or IgG2b-HRP (Caltag).

25     Antibodies, regardless of isotype, were present in the CNS at a concentration that is 1:1000 that found in the blood. For example, when the concentration of IgG1 was three times that of IgG2a in the blood, it was three times IgG2a in the brain as well, both being present at 0.1% of their respective levels in the blood. This result was observed in both transgenic and nontransgenic mice indicating that the PDAPP does not have a

30     uniquely leak blood brain barrier.

## Example VI.   Cloning and Sequencing of the Mouse 3D6 Variable Regions

*Cloning and Sequence Analysis of 3D6 VH.* The heavy chain variable
VH region of 3D6 was cloned by RT-PCR using mRNA prepared from hybridoma cells
by two independent methods. In the first, consensus primers were employed to VH
5     region leader peptide encompassing the translation initiation codon as the 5' primer
(DNA #3818-3829), and a g2b (DNA #3832) constant regions specific 3' primer. The
sequences from PCR amplified product, as well as from multiple, independently-derived
clones, were in complete agreement with one another. As a further check on the
sequence of the 3D6 VH region, the result was confirmed by sequencing a VH fragment
10    obtained by 5' RACE RT-PCR methodology and the 3' g2b specific primer (DNA
#3832). Again, the sequence was derived from the PCR product, as well as multiple,
independently-isolated clones. Both sequences are in complete agreement with one
another, (with the exception of V8I substitution in the leader region from the 5' RACE
product), indicating that the sequences are derived from the mRNA encoding the VH
15    region of 3D6. The nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID
NO:4) of the VH region of 3D6 are set forth in Table 9A and in Figure 2, respectively.


Table 9A:  Mouse 3D6 VH Nucleotide Sequence

<u>ATGAACTTCGGGCTCAGCTTGATTTTCCTTGTCCTTGTTTTAAAAGGTGTCCAGTGTGA</u>
20    <u>AGTGAAGCTGGTGGAGTCTGGGGGAGGCTTAGTGAAGCCTGGAGCGTCTCTGAAACTCT</u>
CCTGTGCAGCCTCTGGATTCACTTTCAGTAACTATGGCATGTCTTGGGTTCGCCAGAAT
TCAGACAAGAGGCTGGAGTGGGTTGCATCCATTAGGAGTGGTGGTGGTAGAACCTACTA
TTCAGACAATGTAAAGGGCCGATTCACCATCTCCAGAGAGAATGCCAAGAACACCCTGT
ACCTGCAAATGAGTAGTCTGAAGTCTGAGGACACGGCCTTGTATTATTGTGTCAGATAT
25    GATCACTATAGTGGTAGCTCCGACTACTGGGGCCAGGGCACCACT  (SEQ ID NO:3)

      *Leader peptide is underlined.


*Cloning and Sequence Analysis of 3D6 VL.* The light chain variable VL
region of 3D6 was cloned in an analogous manner as the VH region. In the first trial, a
consensus primer set was designed for amplification of murine VL regions as follows:
30    5' primers (DNA #3806-3816) were designed to hybridize to the VL region
encompassing the translation initiation codon, and a 3' primer (DNA#3817) was specific

for the murine Ck region downstream of the V-J joining region. DNA sequence analysis

of the PCR fragment, as well as independently-derived clones isolated using this

consensus light chain primer set, revealed that the cDNA obtained was derived from a

non-functionally rearranged message as the sequence contained a frameshift mutation

5    between the V-J region junction.

In a second trial, 5'RACE was employed to clone a second VL encoding

cDNA. DNA sequence analysis of this product (consensus 11) showed it encoded a

functional mRNA. Thus, it can be concluded that the sequence encodes the correct 3D6

light chain mRNA. The nucleotide (SEQ ID NO:1) and amino acid sequence (SEQ ID

10   NO:2) of the VL region of 3D6 are set forth in Table 9B and in Figure 1, respectively.


Table 9B: Mouse 3D6 VL Nucleotide Sequence


ATGATGAGTCCTGCCCAGTTCCTGTTTCTGTTAGTGCTCTGGATTCGGGAAACCAACGG

15   TTATGTTGTGATGACCCAGACTCCACTCACTTTGTCGGTTACCATTGGACAACCAGCCT

CCATCTCTTGCAAGTCAAGTCAGAGCCTCTTAGATAGTGATGGAAAGACATATTTGAAT

TGGTTGTTACAGAGGCCAGGCCAGTCTCCAAAGCGCCTAATCTATCTGGTGTCTAAACT

GGACTCTGGAGTCCCTGACAGGTTCACTGGCAGTGGATCAGGGACAGATTTTACACTGA

AAATCAGCAGAATAGAGGCTGAGGATTTGGGACTTTATTATTGCTGGCAAGGTACACAT

20   TTTCCTCGGACGTTCGGTGGAGGCACCAAGCTGGAAATCAAA    (SEQ ID NO:1)

*Leader peptide is underlined

Primers used for the cloning of the 3D6 VL cDNA are set forth in

Table 10.

| DNA | Size | Coding Strand? | DNA Sequence | Comments |
|-----|------|----------------|--------------|----------|
| 3806 | 40 | Yes | ACT.AGT.CGA.CAT.GAA.GTT.GCC.TGT.TA G.GCT.GTT.GGT.GCT.G (SEQ ID NO:39) | mouse kappa variable primer 1 MKV PRIMER 1, MRC set; % A+T = 50.00 [20]; % C+G = 50.00 [20] Davis,Botstein,Roth Melting Temp C. 72.90 |

| 3807 | 39 | Yes | ACT.AGT.CGA.CAT.GGA.GWC.AGA.CAC.AC<br>T.CCT.GYT.ATG.GGT (SEQ ID NO:40) | mouse kappa variable<br>primer 2<br>MKV PRIMER 2, MRC set<br>% A+T = 46.15 [18]; %<br>C+G = 48.72 [19]<br>Davis,Botstein,Roth<br>Melting Temp C. 72.05 |
|------|----|-----|-----------------------------------|---------------------------------|
| 3808 | 40 | Yes | ACT.AGT.CGA.CAT.GAG.TGT.GCT.CAC.TC<br>A.GGT.CCT.GGS.GTT.G (SEQ ID NO:41) | mouse kappa variable<br>primer 3<br>MKV PRIMER 3, MRC set;<br>% A+T = 45.00 [18]; %<br>C+G = 52.50 [21]<br>Davis,Botstein,Roth<br>Melting Temp C. 73.93 |
| 3809 | 43 | Yes | ACT.AGT.CGA.CAT.GAG.GRC.CCC.TGC.TC<br>A.GWT.TYT.TGG.MWT.CTT.G  (SEQ ID<br>NO:42) | mouse kappa variable<br>primer 4<br>MKV PRIMER 4, MRC set;<br>% A+T = 41.86 [18]; %<br>C+G = 46.51 [20]<br>Davis,Botstein,Roth<br>Melting Temp C. 72.34 |
| 3810 | 40 | Yes | ACT.AGT.CGA.CAT.GGA.TTT.WCA.GGT.GC<br>A.GAT.TWT.CAG.CTT.C  (SEQ ID<br>NO:43) | mouse kappa variable<br>primer 5<br>MKV PRIMER 5, MRC set<br>% A+T = 52.50 [21]; %<br>C+G = 42.50 [17]<br>Davis,Botstein,Roth<br>Melting Temp C. 69.83 |
| 3811 | 37 | Yes | ACT.AGT.CGA.CAT.GAG.GTK.CYY.TGY.TS<br>A.GYT.YCT.GRG.G  (SEQ ID NO:44) | mouse kappa variable<br>primer 6<br>MKV PRIMER 6, MRC set;<br>% A+T = 37.84 [14]; %<br>C+G = 40.54 [15]<br>Davis,Botstein,Roth<br>Melting Temp C. 68.01 |
| 3812 | 41 | Yes | ACT.AGT.CGA.CAT.GGG.CWT.CAA.GAT.GG<br>A.GTC.ACA.KWY.YCW.GG  (SEQ ID<br>NO:45) | mouse kappa variable<br>primer 7<br>MKV PRIMER 7, MRC set;<br>% A+T = 39.02 [16]; %<br>C+G = 46.34 [19]<br>Davis,Botstein,Roth<br>Melting Temp C. 71.70 |
| 3813 | 41 | Yes | ACT.AGT.CGA.CAT.GTG.GGG.AYC.TKT.TT<br>Y.CMM.TTT.TTC.AAT.TG  (SEQ ID<br>NO:46) | mouse kappa variable<br>primer 8<br>MKV PRIMER 8, MRC set;<br>% A+T = 53.66 [22]; %<br>C+G = 34.15 [14]<br>Davis,Botstein,Roth<br>Melting Temp C. 66.70 |

| 3814 | 35 | Yes | ACT.AGT.CGA.CAT.GGT.RTC.CWC.ASC.TC A.GTT.CCT.TG (SEQ ID NO:47) | mouse kappa variable primer 9 MKV PRIMER 9, MRC set. % A+T = 45.71 [16]; % C+G = 45.71 [16] Davis,Botstein,Roth Melting Temp C. 69.36 |
|------|----|-----|-------------------------------------------------------------|-------------------------------------------------------------------------------------------------------------------------|
| 3815 | 37 | Yes | ACT.AGT.CGA.CAT.GTA.TAT.ATG.TTT.GT T.GTC.TAT.TTC.T (SEQ ID NO:48) | mouse kappa variable primer 10 MKV PRIMER 10, MRC set; % A+T = 70.27 [26]; % C+G = 29.73 [11] Davis,Botstein,Roth Melting Temp C. 63.58 |
| 3816 | 38 | Yes | ACT.AGT.CGA.CAT.GGA.AGC.CCC.AGC.TC A.GCT.TCT.CTT.CC (SEQ ID NO:49) | mouse kappa variable primer 11 MKV PRIMER 11, MRC set; % A+T = 44.74 [17]; % C+G = 55.26 [21] Davis,Botstein,Roth Melting Temp C. 74.40 |
| 3817 | 27 | No | GGA.TCC.CGG.GTG.GAT.GGT.GGG.AAG.AT G (SEQ ID NO:50) | mouse kappa light chain reverse primer, aa 116-122; Ck constant region primer, MRC set+SmaI site; % A+T = 47.06 [8]; % C+G = 52.94 [9] Davis,Botstein,Roth Melting Temp C. 57.19 |
| 3818 | 37 | Yes | ACT.AGT.CGA.CAT.GAA.ATG.CAG.CTG.GG T.CAT.STT.CTT.C (SEQ ID NO:51) | mouse heavy variable primer 1 MHV primer 1, MRC set; |
| 3819 | 36 | Yes | ACT.AGT.CGA.CAT.GGG.ATG.GAG.CTR.TA T.CAT.SYT.CTT (SEQ ID NO:52) | mouse heavy variable primer 2 MHV primer 2, MRC set; |
| 3820 | 37 | Yes | ACT.AGT.CGA.CAT.GAA.GWT.GTG.GTT.AA A.CTG.GGT.TTT.T (SEQ ID NO:53) | mouse heavy variable primer 3 MHV primer 3, MRC set; |
| 3821 | 35 | Yes | ACT.AGT.CGA.CAT.GRA.CTT.TGG.GYT.CA G.CTT.GRT.TT (SEQ ID NO:54) | mouse heavy variable primer 4 MHV primer 4, MRC set; |
| 3822 | 40 | Yes | ACT.AGT.CGA.CAT.GGA.CTC.CAG.GCT.CA A.TTT.AGT.TTT.CCT.T (SEQ ID NO:55) | mouse heavy variable primer 5 MHV primer 5, MRC set; |

| 3823 | 37 | Yes | ACT.AGT.CGA.CAT.GGC.TGT.CYT.RGS.GC T.RCT.CTT.CTG.C    (SEQ ID NO:56) | mouse heavy variable primer 6 MHV primer 6, MRC set; |
|------|-----|-----|---------------------------------------|----------------------------------------|
| 3824 | 36 | Yes | ACT.AGT.CGA.CAT.GGR.ATG.GAG.CKG.GR T.CTT.TMT.CTT    (SEQ ID NO:57) | mouse heavy variable primer 7 MHV primer 7, MRC set; |
| 3825 | 33 | Yes | ACT.AGT.CGA.CAT.GAG.AGT.GCT.GAT.TC T.TTT.GTG    (SEQ ID NO:58) | mouse heavy variable primer 8 MHV primer 8, MRC set; |
| 3826 | 40 | Yes | ACT.AGT.CGA.CAT.GGM.TTG.GGT.GTG.GA M.CTT.GCT.ATT.CCT.G    (SEQ ID NO:59) | mouse heavy variable primer 9 MHV primer 9, MRC set; |
| 3827 | 37 | Yes | ACT.AGT.CGA.CAT.GGG.CAG.ACT.TAC.AT T.CTC.ATT.CCT.G    (SEQ ID NO:60) | mouse heavy variable primer 10 MHV primer 10, MRC set; |
| 3828 | 38 | Yes | ACT.AGT.CGA.CAT.GGA.TTT.TGG.GCT.GA T.TTT.TTT.TAT.TG    (SEQ ID NO:61) | mouse heavy variable primer 11 MHV primer 11, MRC set; |
| 3829 | 37 | Yes | ACT.AGT.CGA.CAT.GAT.GGT.GTT.AAG.TC T.TCT.GTA.CCT.G    (SEQ ID NO:62) | mouse heavy variable primer 12 MHV primer 12, MRC set; |
| 3832 | 27 | No | GGA.TCC.CGG.GAG.TGG.ATA.GAC.tGA.TG G    (SEQ ID NO:63) | mouse IgG2b heavy chain reverse primer aa position 119-124, MRC set; |

From N-terminal to C-terminal, both light and heavy chains comprise the domains FR1, CDR1, FR2, CDR2, FR3, CDR3 and FR4. The assignment of amino acids to each domain is in accordance with the numbering convention of Kabat *et al.*, *supra*.

*Expression of Chimeric 3D6 Antibody:* The variable heavy and light chain regions were re-engineered to encode splice donor sequences downstream of the respective VDJ or VJ junctions, and cloned into the mammalian expression vector pCMV-hγ1 for the heavy chain, and pCMV-hκ1 for the light chain. These vectors encode human γ1 and Cк constant regions as exonic fragments downstream of the

inserted variable region cassette. Following sequence verification, the heavy chain and
light chain expression vectors were co-transfected into COS cells. Two different heavy
chain clones (H2.2 & H3.2) were independently co-transfected with 3 different chimeric
light chain clones (L3, L4, &L10) to confirm reproducibility of the result. A chimeric

5    21.6 antibody transfection was carried out as a positive control for the vectors.
Conditioned media was collected 48 hrs post transfection and assayed by western blot
analysis for antibody production or ELISA for Aβ binding.

The multiple transfectants all expressed heavy chain + light chain
combinations which are recognized by a goat anti-human IgG (H+L) antibody on a

10   western blot.

Direct binding of 3D6 and chimeric 3D6 (PK1614) antibodies to Aβ was
tested by ELISA analysis. Chimeric 3D6 was found to bind to Aβ with high avidity,
similar to that demonstrated by 3D6 (Figure 3A). Furthermore, an ELISA based
competitive inhibition assay revealed that the chimeric 3D6 and the murine 3D6

15   antibody competed equally with biotinylated-3D6 binding to Aβ (Figure 3B). The
chimeric antibody displayed binding properties indistinguishable from the 3D6 reference
sample.

Table 11.

| Conc (μg/ml) | 3D6 | PK1614 | IgG1 |
|---|---|---|---|
| 0.037 | | 119.3 | |
| 0.11 | 118.6 | 118.9 | |
| 0.33 | 99.7 | 71.25 | |
| 1 | 98.63 | 84.53 | 134.4 |

20   Moreover, both 3D6 and PK1614 were effective at clearing Aβ plaques.
The *ex vivo* assay demonstrates that as the concentration of antibody increases, the
amount of Aβ decreases in a similar manner for both murine and chimeric 3D6
antibodies. Hence, it can be concluded that the sequences encode functional 3D6 heavy
chain and light chains respectively.

25

### Example VII. 3D6 Humanization

*Homology/Molecular Modeling.* In order to identify key structural
framework residues in the murine 3D6 antibody, a three-dimensional model was

generated based on the closest murine antibodies for the heavy and light chains. For this purpose, an antibody designated 1CR9 was chosen as a template for modeling the 3D6 light chain (PDB ID: 1CR9, Kanyo *et al., supra*), and an antibody designated 1OPG was chosen as the template for modeling the heavy chain. (PDB ID: 1OPG Kodandapani *et*

5      *al., supra*). (See also Table 1.) Amino acid sequence alignment of 3D6 with the light chain and heavy chain of these antibodies revealed that, with the exception of CDR3 of the heavy chain, the 1CR9 and 1OPG antibodies share significant sequence homology with 3D6. In addition, the CDR loops of the selected antibodies fall into the same canonical Chothia structural classes as do the CDR loops of 3D6, again excepting CDR3

10     of the heavy chain. Therefore, 1CR9 and 1OPG were initially selected as antibodies of solved structure for homology modeling of 3D6.

A first pass homology model of 3D6 variable region based on the antibodies noted above was constructed using the Look & SegMod Modules GeneMine (v3.5) software package. This software was purchased under a perpetual license from

15     Molecular Applications Group (Palo Alto, CA). This software package, authored by Drs. Michael Levitt and Chris Lee, facilitates the process of molecular modeling by automating the steps involved in structural modeling a primary sequence on a template of known structure based on sequence homology. Working on a Silicon Graphics IRIS workstation under a UNIX environment, the modeled structure is automatically refined

20     by a series of energy minimization steps to relieve unfavorable atomic contacts and optimize electrostatic and van der Walls interactions.

A further refined model was built using the modeling capability of Quanta®. A query of the PDB database with CDR3 of the heavy chain of 3D6 identified 1qkz as most homologous and having the identical number of residues as 3D6.

25     Hence, CDR3 of the heavy chain of 3D6 was modeled using the crystal structure of 1qkz as template. The α-carbon backbone trace of the 3D6 model is shown in Figure 4. The VH domain is shown as a stippled line, and VL domain is shown as a solid line, and CDR loops are indicated in ribbon form.

30                              *Selection of Human Acceptor Antibody Sequences.* Suitable human acceptor antibody sequences were identified by computer comparisons of the amino acid sequences of the mouse variable regions with the sequences of known human antibodies.

The comparison was performed separately for the 3D6 heavy and light chains. In particular, variable domains from human antibodies whose framework sequences exhibited a high degree of sequence identity with the murine VL and VH framework regions were identified by query of the Kabat Database using NCBI BLAST (publicly

5     accessible through the National Institutes of Health NCBI internet server) with the respective murine framework sequences.

Two candidate sequences were chosen as acceptor sequences based on the following criteria: (1) homology with the subject sequence; (2) sharing canonical CDR structures with the donor sequence; and (3) not containing any rare amino acid

10    residues in the framework regions. The selected acceptor sequence for VL is Kabat ID Number (KABID) 019230 (Genbank Accession No. S40342), and for VH is KABID 045919 (Genbank Accession No. AF115110). First versions of humanized 3D6 antibody utilize these selected acceptor antibody sequences.

15          *Substitution of Amino Acid Residues.* As noted *supra*, the humanized antibodies of the invention comprise variable framework regions substantially from a human immunoglobulin (acceptor immunoglobulin) and complementarity determining regions substantially from a mouse immunoglobulin (donor immunoglobulin) termed 3D6. Having identified the complementarity determining regions of 3D6 and

20    appropriate human acceptor immunoglobulins, the next step was to determine which, if any, residues from these components to substitute to optimize the properties of the resulting humanized antibody. The criteria described *supra* were used to select residues for substitution.

Figures 1 and 2 depict alignments of the original murine 3D6 VL and

25    VH, respectively, with the respective version 1 of the humanized sequence, the corresponding human framework acceptor sequence and, lastly, the human germline V region sequence showing highest homology to the human framework acceptor sequence. The shaded residues indicate the canonical (solid fill), vernier (dotted outline), packing (bold), and rare amino acids (bold italics), and are indicated on the figure. The asterisks

30    indicate residues backmutated to murine residues in the human acceptor framework sequence, and CDR regions are shown overlined. A summary of the changes incorporated into version 1 of humanized 3D6 VH and VL is presented in Table 12.

Table 12. Summary of changes in humanized 3D6.v1

| Changes | VL (112 residues) | VH (119 residues) |
|---|---|---|
| Hu->Mu: Framework | 4/112 | 3/119 (1 canon, 1 packing) |
| CDR1 | 6/16 | 3/5 |
| CDR2 | 4/7 | 7/14 |
| CDR3 | 5/8 | 4/10 |
| Hu->Mu | 19/112 (17%) | 17/119 (14%) |
| Mu->Hu: Framework | 13/112 | 14/119 |
| Backmutation notes | 1. I2V which is a canonical position.<br><br>2. Y36L which is a packing residue and also lies under the CDRs<br><br>3. L46R which is a packing residue and lies beneath the CDRs | 4. S49A Vernier/beneath the CDRs.<br><br>5. A93V which is a packing and vernier zone residue<br><br>6. K94R which is a canonical residue |
| Acceptor notes | 7. KABID 019230/Genbank Acc#S40342<br><br>8. Hu κ LC subgroup II<br><br>9. CDRs from same canonical structural group as donor (m3D6)<br>L1=class 4<br>L2=class 1<br>L3=class1<br><br>10. Unknown specificity | 11. KABID045919/Genbank Acc#AF115110<br><br>12. Hu HC subgroup III<br><br>13. CDRs from same canonical structural group as donor (m3D6)<br>H1=class 1<br>H2=class3<br><br>14. Recognizes capsular polysaccharide of Neisseria meningitidis |
| Acceptor Germline | 15. VH3-23 | 16. A3 & A19 |

Tables 13 and 14 set forth Kabat numbering keys for the various light and

5   heavy chains, respectively.

Table 13: Key to Kabat Numbering for Light Chain

| KAB # | # | TYPE | mouse 3D6 VL | HUM 3D6VL | KABID 019230 | A19-Germ-line | Comment |
|---|---|---|---|---|---|---|---|
| 1 | 1 | FR1 | Y | Y | D | D | Rare mouse, may contact CDR |
| 2 | 2 | | V | V | I | I | Canonical/CDR contact |
| 3 | 3 | | V | V | V | V | |
| 4 | 4 | | M | M | M | M | |
| 5 | 5 | | T | T | T | T | |
| 6 | 6 | | Q | Q | Q | Q | |
| 7 | 7 | | T | S | S | S | |
| 8 | 8 | | P | P | P | P | |
| 9 | 9 | | L | L | L | L | |
| 10 | 10 | | T | S | S | S | |
| 11 | 11 | | L | L | L | L | |
| 12 | 12 | | S | P | P | P | |
| 13 | 13 | | V | V | V | V | |
| 14 | 14 | | T | T | T | T | |
| 15 | 15 | | I | P | P | P | |
| 16 | 16 | | G | G | G | G | |
| 17 | 17 | | Q | E | E | E | |
| 18 | 18 | | P | P | P | P | |
| 19 | 19 | | A | A | A | A | |
| 20 | 20 | | S | S | S | S | |
| 21 | 21 | | I | I | I | I | |
| 22 | 22 | | S | S | S | S | |
| 23 | 23 | | C | C | C | C | |

| 24 | 24 | CDR1 | K | K | R | R | |
|---|---|---|---|---|---|---|---|
| 25 | 25 | | S | S | S | S | |
| 26 | 26 | | S | S | S | S | |
| 27 | 27 | | Q | Q | Q | Q | |
| 27A | 28 | | S | S | S | S | |
| 27B | 29 | | L | L | L | L | |
| 27C | 30 | | L | L | L | L | |
| 27D | 31 | | D | D | H | H | |
| 27E | 32 | | S | S | S | S | |
| 28 | 33 | | D | D | N | N | |
| 29 | 34 | | G | G | G | G | |
| 30 | 35 | | K | K | Y | Y | |
| 31 | 36 | | T | T | N | N | |
| 32 | 37 | | Y | Y | Y | Y | |
| 33 | 38 | | L | L | L | L | |
| 34 | 39 | | N | N | D | D | |
| | | | | | | | |
| 35 | 40 | FR2 | W | W | W | W | |
| 36 | 41 | | L | L | Y | Y | Packing residue |
| 37 | 42 | | L | L | L | L | |
| 38 | 43 | | Q | Q | Q | Q | |
| 39 | 44 | | R | K | K | K | |
| 40 | 45 | | P | P | P | P | |
| 41 | 46 | | G | G | G | G | |
| 42 | 47 | | Q | Q | Q | Q | |
| 43 | 48 | | S | S | S | S | |
| 44 | 49 | | P | P | P | P | |
| 45 | 50 | | K | Q | Q | Q | |
| 46 | 51 | | R | R | L | L | Packing residue |
| 47 | 52 | | L | L | L | L | |
| 48 | 53 | | I | I | I | I | |
| 49 | 54 | | Y | Y | Y | Y | |
| | | | | | | | |
| 50 | 55 | CDR2 | L | L | L | L | |
| 51 | 56 | | V | V | G | G | |
| 52 | 57 | | S | S | S | S | |
| 53 | 58 | | K | K | N | N | |
| 54 | 59 | | L | L | R | R | |
| 55 | 60 | | D | D | A | A | |
| 56 | 61 | | S | S | S | S | |

| 57 | 62 | FR3 | G | G | G | G |
|----|----|-----|---|---|---|---|
| 58 | 63 | | V | V | V | V |
| 59 | 64 | | P | P | P | P |
| 60 | 65 | | D | D | D | D |
| 61 | 66 | | R | R | R | R |
| 62 | 67 | | F | F | F | F |
| 63 | 68 | | T | S | S | S |
| 64 | 69 | | G | G | G | G |
| 65 | 70 | | S | S | S | S |
| 66 | 71 | | G | G | G | G |
| 67 | 72 | | S | S | S | S |
| 68 | 73 | | G | G | G | G |
| 69 | 74 | | T | T | T | T |
| 70 | 75 | | D | D | D | D |
| 71 | 76 | | F | F | F | F |
| 72 | 77 | | T | T | T | T |
| 73 | 78 | | L | L | L | L |
| 74 | 79 | | K | K | K | K |
| 75 | 80 | | I | I | I | I |
| 76 | 81 | | S | S | S | S |
| 77 | 82 | | R | R | R | R |
| 78 | 83 | | I | V | V | V |
| 79 | 84 | | E | E | E | E |
| 80 | 85 | | A | A | A | A |
| 81 | 86 | | E | E | E | E |
| 82 | 87 | | D | D | D | D |
| 83 | 88 | | L | V | V | V |
| 84 | 89 | | G | G | G | G |
| 85 | 90 | | L | V | V | V |
| 86 | 91 | | Y | Y | Y | Y |
| 87 | 92 | | Y | Y | Y | Y |
| 88 | 93 | | C | C | C | C |
| | | | | | | |
| 89 | 94 | CDR3 | W | W | M | M |
| 90 | 95 | | Q | Q | Q | Q |
| 91 | 96 | | G | G | A | A |
| 92 | 97 | | T | T | L | L |
| 93 | 98 | | H | H | Q | Q |
| 94 | 99 | | F | F | T | T |
| 95 | 100 | | P | P | P | P |
| 96 | 101 | | R | R | R | |
| 97 | 102 | | T | T | T | |

| 98   | 103 | FR4 | F | F | F |
|------|-----|-----|---|---|---|
| 99   | 104 |     | G | G | G |
| 100  | 105 |     | G | Q | Q |
| 101  | 106 |     | G | G | G |
| 102  | 107 |     | T | T | T |
| 103  | 108 |     | K | K | K |
| 104  | 109 |     | L | V | V |
| 105  | 110 |     | E | E | E |
| 106  | 111 |     | I | I | I |
| 106A | 112 |     | K | K | K |

Table 14. Key to Kabat Numbering for Heavy Chain

| KAB # | # | TYPE | Mouse 3D6 VH | HUM 3D6 VH | KABID 045919 | VH3-23 Germ-line | Comment |
|---|---|---|---|---|---|---|---|
| 1 | 1 | FR1 | E | E | E | E | |
| 2 | 2 | | V | V | V | V | |
| 3 | 3 | | K | Q | Q | Q | |
| 4 | 4 | | L | L | L | L | |
| 5 | 5 | | V | L | L | L | |
| 6 | 6 | | E | E | E | E | |
| 7 | 7 | | S | S | S | S | |
| 8 | 8 | | G | G | G | G | |
| 9 | 9 | | G | G | G | G | |
| 10 | 10 | | G | G | G | G | |
| 11 | 11 | | L | L | L | L | |
| 12 | 12 | | V | V | V | V | |
| 13 | 13 | | K | Q | Q | Q | |
| 14 | 14 | | P | P | P | P | |
| 15 | 15 | | G | G | G | G | |
| 16 | 16 | | A | G | G | G | |
| 17 | 17 | | S | S | S | S | |
| 18 | 18 | | L | L | L | L | |
| 19 | 19 | | K | R · | R | R | |
| 20 | 20 | | L | L | L | L | |
| 21 | 21 | | S | S | S | S | |
| 22 | 22 | | C | C | C | C | |
| 23 | 23 | | A | A | A | A | |
| 24 | 24 | | A | A | A | A | |
| 25 | 25 | | S | S | S | S | |
| 26 | 26 | | G | G | G | G | |
| 27 | 27 | | F | F | F | F | |
| 28 | 28 | | T | T | T | T | |
| 29 | 29 | | F | F | F | F | |
| 30 | 30 | | S | S | S | S | |
| 31 | 31 | CDR1 | N | N | S | S | |
| 32 | 32 | | Y | Y | Y | Y | |
| 33 | 33 | | G | G | A | A | |
| 34 | 34 | | M | M | V | M | |
| 35 | 35 | | S | S | S | S | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 36 | 36 | FR2 | W | W | W | W | |
| 37 | 37 | | V | V | V | V | |
| 38 | 38 | | R | R | R | R | |
| 39 | 39 | | Q | Q | Q | Q | |
| 40 | 40 | | N | A | A | A | Rare mouse, replace w/Hum |
| 41 | 41 | | S | P | P | P | |
| 42 | 42 | | D | G | G | G | Rare mouse, replace w/Hum |
| 43 | 43 | | K | K | K | K | |
| 44 | 44 | | R | G | G | G | |
| 45 | 45 | | L | L | L | L | |
| 46 | 46 | | E | E | E | E | |
| 47 | 47 | | W | W | W | W | |
| 48 | 48 | | V | V | V | V | |
| 49 | 49 | | A | A | S | S | CDR contact/veneer |
| | | | | | | | |
| 50 | 50 | CDR2 | S | S | A | A | |
| 51 | 51 | | I | I | I | I | |
| 52 | 52 | | R | R | S | S | |
| 52A | 53 | | S | S | G | G | |
| 53 | 54 | | G | G | S | S | |
| 54 | 55 | | G | G | G | G | |
| 55 | 56 | | G | G | G | G | |
| 56 | 57 | | R | R | S | S | |
| 57 | 58 | | T | T | T | T | |
| 58 | 59 | | Y | Y | Y | Y | |
| 59 | 60 | | Y | Y | Y | Y | |
| 60 | 61 | | S | S | A | A | |
| 61 | 62 | | D | D | D | D | |
| 62 | 63 | | N | N | S | S | |
| 63 | 64 | | V | V | V | V | |
| 64 | 65 | | K | K | K | K | |
| 65 | 66 | | G | G | G | G | |

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| 66 | 67 | FR3 | R | R | R | R | |
| 67 | 68 | | F | F | F | F | |
| 68 | 69 | | T | T | T | T | |
| 69 | 70 | | I | I | I | I | |
| 70 | 71 | | S | S | S | S | |
| 71 | 72 | | R | R | R | R | |
| 72 | 73 | | E | D | D | D | |
| 73 | 74 | | N | N | N | N | |
| 74 | 75 | | A | A | A | S | |
| 75 | 76 | | K | K | K | K | |
| 76 | 77 | | N | N | N | N | |
| 77 | 78 | | T | S | S | T | |
| 78 | 79 | | L | L | L | L | |
| 79 | 80 | | Y | Y | Y | Y | |
| 80 | 81 | | L | L | L | L | |
| 81 | 82 | | Q | Q | Q | Q | |
| 82 | 83 | | M | M | M | M | |
| 82A | 84 | | S | N | N | N | |
| 82B | 85 | | S | S | S | S | |
| 82C | 86 | | L | L | L | L | |
| 83 | 87 | | K | R | R | R | |
| 84 | 88 | | S | A | A | A | |
| 85 | 89 | | E | E | E | E | |
| 86 | 90 | | D | D | D | D | |
| 87 | 91 | | T | T | T | T | |
| 88 | 92 | | A | A | A | A | |
| 89 | 93 | | L | L | L | V | |
| 90 | 94 | | Y | Y | Y | Y | |
| 91 | 95 | | Y | Y | Y | Y | |
| 92 | 96 | | C | C | C | C | |
| 93 | 97 | | V | V | A | A | Packing residue, use mouse |
| 94 | 98 | | R | R | K | K | Canonical, use mouse |
| | | | | | | | |
| 95 | 99 | CDR3 | Y | Y | D | | |
| 96 | 100 | | D | D | N | | |
| 97 | 101 | | H | H | Y | | |
| 98 | 102 | | Y | Y | D | | |
| 99 | 103 | | S | S | F | | |
| 100 | 104 | | G | G | W | | |
| 100A | 105 | | S | S | S | | |
| 100B | 106 | | S | S | G | | |
| 100C | 107 | | - | - | T | | |
| 100D | 108 | | - | - | F | | |
| 101 | 109 | | D | D | D | | |
| 102 | 110 | | Y | Y | Y | | |

| 103 | 111 | FR4 | W | W | W |
|-----|-----|-----|---|---|---|
| 104 | 112 |     | G | G | G |
| 105 | 113 |     | Q | Q | Q |
| 106 | 114 |     | G | G | G |
| 107 | 115 |     | T | T | T |
| 108 | 116 |     | T | L | L |
| 109 | 117 |     | V | V | V |
| 110 | 118 |     | T | T | T |
| 111 | 119 |     | V | V | V |
| 112 | 120 |     | S | S | S |
| 113 | 121 |     | S | S | S |

The humanized antibodies preferably exhibit a specific binding affinity for Aβ of at least $10^7$, $10^8$, $10^9$ or $10^{10}$ $M^{-1}$. Usually the upper limit of binding affinity of the humanized antibodies for Aβ is within a factor of three, four or five of that of 3D6 (*i.e.*, ~$10^9$ $M^{-1}$). Often the lower limit of binding affinity is also within a factor of three, four or five of that of 3D6.

*Assembly and Expression of Humanized 3D6 VH and VL, Version 1*

Briefly, for each V region, 4 large single stranded overlapping oligonucleotides were synthesized. In addition, 4 short PCR primers were synthesized for each V region to further facilitate assembly of the particular V region. The DNA sequences of the oligonucleotides employed for this purpose are shown in Table 15.

Table 15: DNA oligonucleotides

| DNA# | SIZE | Coding? | Sequence | comments |
|------|------|---------|----------|----------|
| 4060 | 136 | Yes | tccgc aagct tgccg ccacc ATGGA CATGC GCGTG CCCGC CCAGC TGCTG GGCCT GCTGA TGCTG TGGGT GTCCG GCTCC TCCGG CTACG TGGTG ATGAC CCAGT CCCCC CTGTC CCTGC CCGTG ACCCC CGGCG A (SEQ ID NO:17) | hum 3D6 VL-A |
| 4061 | 131 | No | CTGGG GGGAC TGGCC GGGCT TCTGC AGCAG CCAGT TCAGG TAGGT CTTGC CGTCG GAGTC CAGCA GGGAC TGGGA GGACT TGCAG GAGAT GGAGG CGGGC TCGCC GGGGG TCACG GCAG GGACA GGGGG G (SEQ ID NO:18) | hum 3D6 VL-B |

| 4062 | 146 | Yes | ACCTG AACTG GCTGC TGCAG AAGCC CGGCC AGTCC CCCCA GCGCC TGATC TACCT GGTGT CCAAG CTGGA CTCCG GCGTG CCCGA CCGCT TCTCC GGCTC CGGCT CCGGC ACCGA CTTCA CCCTG AAGAT CTCCC GCGTG GAGGC C    (SEQ ID NO:19) | hum 3D6 VL-C |
|------|-----|-----|-----|-----|
| 4063 | 142 | No | aattc tagga tccac tcacg CTTGA TCTCC ACCTT GGTGC CCTGG CCGAA GGTGC GGGGG AAGTG GGTGC CCTGC CAGCA GTAGT ACACG CCCAC GTCCT CGGCC TCCAC GCGGG AGATC TTCAG GGTGA AGTCG GTGCC GG    (SEQ ID NO:20) | hum 3D6 VL-D |
| 4064 | 16 | No | CTGGG GGGAC TGGCC G (SEQ ID NO: 21) | hum 3D6 VL A+B back %A+T = 18.75 [3]; % C+G = 81.2[13] Davis,Botstein,Roth Melting Temp C. 66.96 |
| 4065 | 22 | Yes | ACCTG AACTG GCTGC TGCAG AA    (SEQ ID NO:22) | hum 3D6 VL C+D forward % A+T = 45.45 [10]; % C+G = 54.55 [12] Davis,Botstein,Roth Melting Temp C. 64.54 |
| 4066 | 138 | Yes | acaga aagct tgccg ccacc ATGGA GTTTG GGCTG AGCTG GCTTT TTCTT GTGGC TATTT TAAAA GGTGT CCAGT GTGAG GTGCA GCTGC TGGAG TCCGG CGGCG GCCTG GTGCA GCCCG GCGGC TCCCT GCGCC TGT (SEQ ID NO:23) | hum 3D6 VH-A |
| 4067 | 135 | No | GCCGC CGGAG CGGAT GGAGG CCACC CACTC CAGGC CCTTG CCGGG GGCCT GGCGC ACCCA GGACA TGCCG TAGTT GGAGA AGGTG AAGCC GGAGG CGGCG CAGGA CAGGC GCAGG GAGCC GCCGG GCTGC ACCAG (SEQ ID NO:24) | hum 3D6 VH-B |
| 4068 | 142 | Yes | CTGGA GTGGG TGGCC TCCAT CCGCT CCGGC GGCGG CCGCA CCTAC TACTC CGACA ACGTG AAGGG CCGCT TCACC ATCTC CCGCG ACAAC GCCAA GAACT CCCTG TACCT GCAGA TGAAC TCCCT GCGCG CCGAG GACAC CG  (SEQ ID NO:25) | hum 3D6 VH-C |

| 4069 | 144 | No | ctgca aggat ccact caccG GAGGA CACGG TCACC AGGGT GCCCT GGCCC CAGTA GTCGG AGGAG CCGGA GTAGT GGTCG TAGCG CACGC AGTAG TACAG GGCGG TGTCC TCGGC GCGCA GGGAG TTCAT CTGCA GGTAC AGGG (SEQ ID NO:26) | hum 3D6 VH-D |
|------|-----|-----|------|------|
| 4070 | 16 | No | GCCGC CGGAG CGGAT G (SEQ ID NO:27) | hum 3D6 VH A+B back % A+T = 18.75 [3]; % C+G = 81.25[13] Davis,Botstein,Roth Melting Temp C. 66.96 |
| 4071 | 20 | Yes | CTGGA GTGGG TGGCC TCCAT (SEQ ID NO:28) | hum 3D6 VH C+D forward % A+T = 35.00 [7]; % C+G = 65.00 [13] Davis,Botstein,Roth Melting Temp C. 66.55 |
| 4072 | 19 | Yes | tcc gca agc ttg ccg cca c (SEQ ID NO:29) | Hum 3D6 VL A+B Forward % A+T = 31.58 [6]; % C+G = 68.42[13] Davis,Botstein,Roth Melting Temp C. 66.64 |
| 4073 | 29 | No | aat tct agg atc cac tca cgC TTG ATC TC (SEQ ID NO:30) | Hum 3D6 VL C+D Back % A+T = 55.17[16]; % C+G = 44.83 [13] Davis,Botstein,Roth Melting Temp C. 66.04 |
| 4074 | 23 | Yes | aca gaa agc ttg ccg cca ccA TG (SEQ ID NO:31) | Hum 3D6 VH A+B Forward % A+T = 43.48 [10]; % C+G = 56.52 [13] Davis,Botstein,Roth Melting Temp C. 66.33 |
| 4075 | 22 | No | ctg caa gga tcc act cac cGG A (SEQ ID NO:32) | Hum 3D6 VH C+D Back % A+T = 40.91 [9]; % C+G = 59.09[13] Davis,Botstein,Roth Melting Temp C. 66.40 |

The humanized light chain was assembled using PCR. DNA sequence
analysis of greater than two dozen clones revealed scattered point mutations and
deletions throughout the VL region with respect to the expected sequence. Analysis of
the sequences indicated that clone 2.3 was amenable to repair of 2 closely spaced single

5      nucleotide deletions in the amino-terminal region. Hence site directed mutagenesis was
performed on clone pCRShum3D6v12.3 using oligonucleotides to introduce the 2
deleted nucleotides, and repair of the point mutations was confirmed by DNA sequence
analysis, and the VL insert was cloned into the light chain expression vector pCMV-cK.

Assembly of humanized VH using PCR-based methods resulted in clones

10     with gross deletions in the 5' half of the sequence. Further efforts to optimize the PCR
conditions met with partial success. The clones assembled via optimized PCR
conditions still had 10-20 nt deletions in the region mapping to the overlap of the A+B
fragments. Consequently, an alternate strategy was employed for VH assembly utilizing
DNA polymerase (T4, Klenow, and Sequenase) mediated overlap extension, followed

15     by T4 DNA ligase to covalently join the overlapping ends. DNA sequence analysis of a
subset of the clones resulting from VH assembly using the latter approach revealed
scattered point mutations and deletions among the clones. Analysis of over two dozen
clones revealed essentially the same pattern as illustrated for the clones. The similar
results observed following first pass assembly of VH and VL clones suggests the DNA

20     sequence errors observed resulted from automated synthesizer errors during the
synthesis of the long DNAs employed for the assembly.

Humanized VH clone 2.7 was selected for site-directed mutagenesis-
mediated repair of the 3 nucleotide deletions it was observed to contain.


25     **Example XIII: Characterization of Humanized 3D6v2 Antibody**

A second version of humanized 3D6 was created having each of the
substitutions indicated for version 1, except for the D→ Y substitution at residue 1.
Substitution at this residue was performed in version 1 because the residue was
identified as a CDR interacting residue. However, substitution deleted a residue which

30     was rare for human immunoglobulins at that position. Hence, a version was created
without the substitution. Moreover, non-germline residues in the heavy chain
framework regions were substituted with germline residues, namely, H74 = S, H77 = T

and H89 = V. Kabat numbering for the version 2 light and heavy chains, is the same as that depicted in Tables 13 and 14, respectively, except that residue 1 of the version 2 light chain is asp (D), residue 74 of the heavy chain is ser (S), residue 77 of the heavy chain is thr (T) and residue 89 of the heavy chain is val (V). The nucleotide sequence of

5    humanized 3D6 version 1 light and heavy chains are set forth as SEQ ID NOs: 34 and 36, respectively. The nucleotide sequence of humanized 3D6 version 2 light and heavy chains are set forth as SEQ ID NOs: 35 and 37, respectively.


### Example IX:  Functional Testing of Humanized 3D6 Antibodies

10    *Binding of humanized 3D6v1 to aggregated Aβ.* Functional testing of humanized 3D6v1 was conducted using conditioned media from transiently transfected COS cells. The cells were transfected with fully chimeric antibody, a mixture of either chimeric heavy chain + humanized light chain, or chimeric light chain + humanized heavy chain, and lastly, fully humanized antibody. The conditioned media was tested

15    for binding to aggregated Aβ1-42 by ELISA assay. The humanized antibody showed good activity within experimental error, and displayed binding properties indistinguishable from the chimeric 3D6 reference sample. The results are shown in Table 16.

Table 16:

| ng/ml | Chimeric | hu VH/ ChVL | ChVH/ HuVL | Hu VH/ HuVL |
|---|---|---|---|---|
| 690 | | | 0.867 | |
| 600 | | | | 0.895 |
| 260 | 0.83 | | | |
| 230 | | | 0.774 | |
| 200 | | | | 0.81 |
| 190 | | 0.811 | | |
| 87 | 0.675 | | | |
| 77 | | | 0.594 | |
| 67 | | | | 0.689 |
| 63 | | 0.648 | | |
| 29 | 0.45 | | | |
| 25 | | | 0.381 | |
| 22 | | | | 0.496 |
| 21 | | 0.438 | | |
| 9.6 | 0.251 | | | |
| 8.5 | | | 0.198 | |
| 7.4 | | | | 0.278 |
| 7 | | 0.232 | | |
| 3.2 | 0.129 | | | |
| 2.3 | | 0.124 | | |

To compare the binding affinities of humanized 3D6v1 and 3D6v2 antibodies, ELISA analysis was performed using aggregated Aβ as the antigen. The results show that both 3D6v1 (H1L1) and 3D6v2 (H2L2) have nearly identical Aβ binding properties (Figure 5).

*Replacement NET (rNET) analysis of h3D6v2.* The rNET epitope map assay provides information about the contribution of individual residues within the epitope to the overall binding activity of the antibody. rNET analysis uses synthesized systematic single substituted peptide analogs. Binding of an antibody being tested is determined against native peptide (native antigen) and against 19 alternative "single substituted" peptides, each peptide being substituted at a first position with one of 19 non-native amino acids for that position. A profile is generated reflecting the effect of substitution at that position with the various non-native residues. Profiles are likewise generated at successive positions along the antigenic peptide. The combined profile, or epitope map, (reflecting substitution at each position with all 19 non-native residues) can then be compared to a map similarly generated for a second antibody. Substantially similar or identical maps indicate that antibodies being compared have the same or similar epitope specificity.

This analysis was performed for 3D6 and humanized 3D6, version 2. Antibodies were tested for binding against the native Aβ peptide DAEFRHDSGY (SEQ ID NO:33). Residues 1-8 were systematically substituted with each of the 19 non-native residues for that position. Maps were generated accordingly for 3D6 and h3D6v2. The results are presented in tabular form in Table 17.

Table 17: Aβ: replacement Net Epitope (rNET) mapping of wt3D6 and humanized 3D6

| Substitution | Wildtype 3D6 [OD] | Humanized 3D6 [OD] | Substitution | Wildtype 3D6 [OD] | Humanized 3D6 [OD] |
|---|---|---|---|---|---|
| Residue 1 = A | 0.464 | 0.643 | Residue 5 = A | 0.275 | 0.435 |
| C | 0.450 | 0.628 | C | 0.359 | 0.635 |
| D | 0.577 | 0.692 | D | 0.080 | 0.163 |
| E | 0.576 | 0.700 | E | 0.115 | 0.187 |
| F | 0.034 | 0.062 | F | 0.439 | 0.569 |
| G | 0.569 | 0.738 | G | 0.485 | 0.679 |
| H | 0.054 | 0.117 | H | 0.577 | 0.680 |
| I | 0.048 | 0.118 | I | 0.510 | 0.671 |
| K | 0.033 | 0.057 | K | 0.573 | 0.693 |
| L | 0.073 | 0.148 | L | 0.517 | 0.691 |
| M | 0.039 | 0.072 | M | 0.418 | 0.611 |
| N | 0.587 | 0.757 | N | 0.476 | 0.655 |
| P | 0.069 | 0.144 | P | 0.093 | 0.198 |
| Q | 0.441 | 0.689 | Q | 0.388 | 0.565 |
| R | 0.056 | 0.155 | R | 0.613 | 0.702 |
| S | 0.569 | 0.762 | S | 0.487 | 0.633 |
| T | 0.450 | 0.702 | T | 0.530 | 0.639 |
| V | 0.057 | 0.190 | V | 0.493 | 0.562 |
| W | 0.031 | 0.070 | W | 0.393 | 0.461 |
| Y | 0.341 | 0.498 | Y | 0.278 | 0.230 |
| Residue 2 = A | 0.548 | 0.698 | Residue 6 = A | 0.587 | 0.707 |
| C | 0.553 | 0.694 | C | 0.585 | 0.703 |
| D | 0.119 | 0.222 | D | 0.584 | 0.701 |
| E | 0.563 | 0.702 | E | 0.579 | 0.702 |
| F | 0.577 | 0.717 | F | 0.586 | 0.704 |
| G | 0.527 | 0.720 | G | 0.592 | 0.709 |
| H | 0.534 | 0.741 | H | 0.596 | 0.688 |
| I | 0.522 | 0.722 | I | 0.602 | 0.708 |
| K | 0.548 | 0.722 | K | 0.585 | 0.691 |
| L | 0.482 | 0.705 | L | 0.584 | 0.688 |
| M | 0.535 | 0.705 | M | 0.583 | 0.687 |
| N | 0.525 | 0.735 | N | 0.580 | 0.686 |
| P | 0.445 | 0.707 | P | 0.587 | 0.705 |
| Q | 0.567 | 0.756 | Q | 0.570 | 0.695 |
| R | 0.562 | 0.719 | R | 0.576 | 0.686 |

| | | | | | |
|---|---|---|---|---|---|
| S | 0.587 | 0.705 | S | 0.573 | 0.689 |
| T | 0.552 | 0.712 | T | 0.573 | 0.700 |
| V | 0.550 | 0.702 | V | 0.588 | 0.715 |
| W | 0.553 | 0.701 | W | 0.576 | 0.696 |
| Y | 0.547 | 0.704 | Y | 0.595 | 0.708 |
| Residue 3 = A | 0.038 | 0.061 | Residue 7 = A | 0.580 | 0.688 |
| C | 0.222 | 0.410 | C | 0.559 | 0.676 |
| D | 0.019 | 0.027 | **D** | **0.573** | **0.681** |
| **E** | **0.542** | **0.689** | E | 0.565 | 0.677 |
| F | 0.034 | 0.060 | F | 0.546 | 0.668 |
| G | 0.016 | 0.019 | G | 0.562 | 0.679 |
| H | 0.016 | 0.020 | H | 0.557 | 0.675 |
| I | 0.019 | 0.024 | I | 0.552 | 0.681 |
| K | 0.053 | 0.090 | K | 0.565 | 0.685 |
| L | 0.019 | 0.026 | L | 0.566 | 0.701 |
| M | 0.019 | 0.027 | M | 0.562 | 0.697 |
| N | 0.024 | 0.032 | N | 0.573 | 0.688 |
| P | 0.017 | 0.020 | P | 0.582 | 0.678 |
| Q | 0.153 | 0.406 | Q | 0.563 | 0.679 |
| R | 0.015 | 0.023 | R | 0.551 | 0.677 |
| S | 0.016 | 0.021 | S | 0.563 | 0.674 |
| T | 0.015 | 0.019 | T | 0.560 | 0.685 |
| V | 0.016 | 0.021 | V | 0.563 | 0.687 |
| W | 0.149 | 0.304 | W | 0.547 | 0.685 |
| Y | 0.016 | 0.020 | Y | 0.560 | 0.682 |
| Residue 4 = A | 0.016 | 0.020 | Residue 8 = A | 0.573 | 0.687 |
| C | 0.020 | 0.023 | C | 0.583 | 0.700 |
| D | 0.017 | 0.020 | D | 0.586 | 0.697 |
| E | 0.016 | 0.021 | E | 0.601 | 0.701 |
| **F** | **0.557** | **0.703** | F | 0.586 | 0.687 |
| G | 0.016 | 0.020 | G | 0.569 | 0.681 |
| H | 0.470 | 0.723 | H | 0.559 | 0.683 |
| I | 0.119 | 0.360 | I | 0.568 | 0.686 |
| K | 0.015 | 0.018 | K | 0.557 | 0.698 |
| L | 0.559 | 0.716 | L | 0.570 | 0.686 |
| M | 0.549 | 0.725 | M | 0.571 | 0.693 |
| N | 0.085 | 0.089 | N | 0.573 | 0.700 |
| P | 0.030 | 0.056 | P | 0.574 | 0.694 |
| Q | 0.065 | 0.110 | Q | 0.590 | 0.703 |
| R | 0.016 | 0.019 | R | 0.589 | 0.699 |
| S | 0.026 | 0.031 | **S** | **0.599** | **0.719** |
| T | 0.016 | 0.021 | T | 0.586 | 0.689 |
| V | 0.213 | 0.494 | V | 0.578 | 0.688 |
| W | 0.291 | 0.568 | W | 0.567 | 0.687 |
| Y | 0.529 | 0.730 | Y | 0.574 | 0.680 |

Notably, the profiles are virtually identical for 3D6 and h3D6v2 when one looks at the substitutions at each position (*i.e.*, the values fluctuate in an identical

manner when comparing the data in column 1 (3D6) versus column 2 (h3D6v2). These data demonstrate that the specificity of h3D6v2 is preserved, as the h3D6v2 rNET epitope map is virtually identical to m3D6 using both Aβ residues 1-4 and 5-8.

5    *Immunohistochemistry on PDAPP brain sections demonstrates specificity of h3D6v1 antibody.* Humanized 3D6v1 antibody recognized Aβ in cryostat prepared brain sections from PDAPP mice. Humanized 3D6v1 and PK1614 both bound to PDAPP plaques in the same dose response fashion, as measured by the amount of fluorescence (quantitated in pixels) per slide versus the amount of antibody used to stain the tissue (Figure 6). Identical anti-human secondary antibodies were used in this

10   experiment. Sectioning, staining, and image procedures were previously described. In identical experiments, image analysis of h3D6v2 staining on PDAPP and AD brain sections revealed that h3D6v2 recognizes Aβ plaques in a similar manner to 3D6v1 (*e.g.*, highly decorated plaques).

*Competitive binding analysis of h3D6.* The ability of h3D6 antibodies v1

15   and v2 to compete with murine 3D6 was measured by ELISA using a biotinylated 3D6 antibody. Competitive binding analysis revealed that h3D6v1, h3D6v2, and chimeric PK1614 can all compete with m3D6 to bind Aβ (Figure 7). h3D6v1 and h3D6v2 were identical in their ability to compete with 3D6 to Aβ. The 10D5 antibody was used as a negative control, as it has a different binding epitope than 3D6. BIAcore analysis also

20   revealed a high affinity of h3D6v1 and h3D6v2 for Aβ (Table 18).

Table 18: Affinity Measurements of Aβ Antibodies Using BIAcore Technology

| Antibody | ka1 (1/Ms) | kd1 (1/s) | Kd (nM) |
|---|---|---|---|
| Mu 3D6 | 4.06E +05 | 3.57E-04 | 0.88 |
| Chimeric 3D6 | 4.58E+05 | 3.86E-04 | 0.84 |
| Hu 3D6v1 | 1.85E+05 | 3.82E-04 | 2.06 |
| Hu 3D6v2 | 1.70E+05 | 3.78E-04 | 2.24 |

25       In comparison to 3D6, which has a Kd of 0.88 nM, both h3D6v1 and h3D6v2 had about a 2 to 3 fold less binding affinity, measured at 2.06 nM and 2.24 nM for h3D6v1 and h3D6v2, respectively. The ELISA competitive binding assay revealed

an approximate 6-fold less binding affinity for h3D6v1 and h3D6v2. Typically humanized antibodies lose about 3-4 fold in binding affinity in comparison to their murine counterparts. Therefore, a loss of about 3 fold (average of ELISA and BIAcore results) for h3D6v1 and h3D6v2 is within the accepted range.

5          *Ex vivo assay using h3D6v2 antibody.* The ability of h3D6v2 to stimulate microglial cells was tested through an *ex vivo* phagocytosis assay (Figure 8). h3D6v2 was as effective as chimeric 3D6 at inducing phagocytosis of Aβ aggregates from PDAPP mouse brain tissue. IgG was used as a negative control in this experiment because it is incapable of binding Aβ and therefore cannot induce phagocytosis.

10         *In vivo brain localization of h3D6.* $^{125}$I labeled h3D6v2, m3D6, and antibody DAE13 were each IV-injected into 14 individual PDAPP mice in separate experiments. Mice were sacrificed after Day 7 and perfused for further analysis. Their brain regions were dissected and measured for $^{125}$I activity in specific brain regions. Radiolabel activity in the brain was compared with activity in serum samples. Results

15    are set forth in Tables 19 and 20, for serum and brain regions, respectively.

Table 19

| m3D6 | DAE13 | Hu3D6 |
|---|---|---|
| 30389.1 | 17463.9 | 40963.8 |
| 12171 | 13200.6 | 24202.2 |
| 3418.2 | 36284.7 | 12472.4 |
| 18678.9 | 421.3 | 33851.8 |
| 27241 | 19702 | 27187.3 |
| 26398.8 | 24855.8 | 29016.9 |
| 27924.8 | 29287.4 | 33830.7 |
| 12008.4 | 12733.1 | 26734.9 |
| 29487.8 | 27722.5 | 30144.5 |
| 25498.6 | 30460.7 | 35126.9 |
| 9652 | 23320.1 | 28414.8 |
| 24599.3 | 7119.1 | 16956.1 |
| 29240 | 28093.5 | 18190.7 |
| 11922.7 | 24659.7 | 25671.4 |
| 17443.1 | 26748.9 | |

Table 20

| m3D6 | | | DAE13 | | | Hu3D6 (H2L2) | | |
|------|------|------|------|------|------|------|------|------|
| cere | cort | hipp | cere | cort | hipp | cere | cort | hipp |
| 1991.9 | 1201.1 | 4024 | 1277.5 | 2522.9 | 5711.9 | 2424.6 | 3759.4 | 11622 |
| 238.9 | 746.1 | 2523 | 502.5 | 2123.5 | 6965.8 | 1509.8 | 2274.9 | 7018.2 |
| 645.9 | 603 | 1241.1 | 2325 | 3528.2 | 7801.6 | 500 | 2265.9 | 5316.3 |
| 1000 | 2508.2 | 4644.2 | 232.7 | 849.8 | 1891.9 | 2736.2 | 5703.7 | 10395.5 |
| 1266.9 | 3737.9 | 7975.8 | 891.6 | 2621 | 8245.2 | 1192.2 | 3188 | 10170 |
| 1422 | 2398.7 | 7731.1 | 1102.6 | 2087.5 | 7292.3 | 2269.4 | 3481.4 | 9621.6 |
| 1700.4 | 2154.4 | 7124.1 | 1650.6 | 3488.4 | 10284.8 | 1526.7 | 3028 | 8331.3 |
| 542.5 | 812.4 | 2456.8 | 712.9 | 2318.5 | 6643.3 | 1538.1 | 4194.1 | 11244.8 |
| 1309 | 3010.5 | 8693.5 | 1172.9 | 1953.6 | 7363 | 1245.7 | 1699.4 | 6831.2 |
| 1372.2 | 997.5 | 2425.4 | 1067.9 | 3697.2 | 12280.7 | 2708.8 | 2789 | 7887.4 |
| 778.6 | 1291.9 | 5654.4 | 1952.2 | 2120.7 | 6412.7 | 2251.3 | 3897.5 | 11121.5 |
| 1199.3 | 1683.4 | 4887.3 | 1005.2 | 1852.5 | 5121.4 | 1529.6 | 1772.2 | 7986.9 |
| 1021.8 | 3234.5 | 8036.2 | 961.5 | 3382.9 | 8473.1 | 644.1 | 1663.4 | 5056.5 |
| 742.1 | 1056.7 | 3405.2 | 852.3 | 1943.2 | 6717.4 | 1516.4 | 1620.6 | 9888 |
| 1273.7 | 1320.8 | 4262.6 | 997.5 | 3065.7 | 10213.1 | | | |

The data show that h3D6v2 localized to the brain, and was particularly concentrated in the hippocampal region where Aβ is known to aggregate. Brain counts for m3D6 and DAE13 were comparable to h3D6v2. All three antibodies were able to cross the blood barrier as demonstrated by Aβ plaque binding *in vivo*.

## Example X. Cloning and Sequencing of the Mouse 10D5 Variable Regions

*Cloning and Sequence Analysis of 10D5 VH.* The VH and VL regions of 10D5 from hybridoma cells were cloned by RT-PCR using 5' RACE procedures. The nucleotide sequence (SEQ ID NO:13) and deduced amino acid sequence (SEQ ID NO:14) derived from two independent cDNA clones encoding the presumed 10D5 VL domain, are set forth in Table 21 and Figure 9. The nucleotide sequence (SEQ ID NO:15) and deduced amino acid sequence (SEQ ID NO:16) derived from two independent cDNA clones encoding the presumed 10D5 VH domain, are set forth in Table 22 and Figure 10. The 10D5 VL and VH sequences meet the criteria for functional V regions in so far as they contain a contiguous ORF from the initiator methionine to the C-region, and share conserved residues characteristic of immunoglobulin V region genes.

Table 21: Mouse 10D5 VL DNA sequence


<u>ATGAAGTTGCCTGTTAGGCTGTTGGTACTGATGTTCTGGATTCCTGCTTCCAGCAGTGA</u>
TGTTTTGATGACCCAAACTCCACTCTCCCTGCCTGTCAGTCTTGGAGATCAAGCCTCCA
5   TCTCTTGCAGATCTAGTCAGAACATTATACATAGTAATGGAAACACCTATTTAGAATGG
TACCTGCAGAAACCAGGCCAGTCTCCAAAGCTCCTGATCTACAAAGTTTCCAACCGATT
TTCTGGGGTCCCAGACAGGTTCAGTGGCAGTGGATCAGGGACAGATTTCACACTCAAGA
TCAAGAAAGTGGAGGCTGAGGATCTGGGAATTTATTACTGCTTTCAAGGTTCACATGTT
CCGCTCACGTTCGGTGCTGGGACCAAGCTGGAGCTGGAA     (SEQ ID NO:13)

10   *Leader peptide underlined


Table 22: Mouse 10D5 VH DNA sequence.


<u>ATGGACAGGCTTACTTCCTCATTCCTGCTGCTGATTGTCCCTGCATATGTCCTGTCCCA</u>
15   GGCTACTCTGAAAGAGTCTGGCCCTGGAATATTGCAGTCCTCCCAGACCCTCAGTCTGA
CTTGTTCTTTCTCTGGGTTTTCACTGAGCACTTCTGGTATGGGAGTGAGCTGGATTCGT
CAGCCTTCAGGAAAGGGTCTGGAGTGGCTGGCACACATTTACTGGGATGATGACAAGCG
CTATAACCCATCCCTGAAGAGCCGGCTCACAATCTCCAAGGATACCTCCAGAAAGCAGG
TATTCCTCAAGATCACCAGTGTGGACCCTGCAGATACTGCCACATACTACTGTGTTCGA
20   AGGCCCATTACTCCGGTACTAGTCGATGCTATGGACTACTGGGGTCAAGGAACCTCAGT
CACCGTCTCCTCA     (SEQ ID NO:15)

*Leader peptide underlined.


## Example XI.  Prevention and Treatment of Human Subjects

25            A single-dose phase I trial is performed to determine safety in humans.  A
therapeutic agent is administered in increasing dosages to different patients starting from
about 0.01 the level of presumed efficacy, and increasing by a factor of three until a
level of about 10 times the effective mouse dosage is reached.

            A phase II trial is performed to determine therapeutic efficacy.  Patients
30   with early to mid Alzheimer's Disease defined using Alzheimer's disease and Related
Disorders Association (ADRDA) criteria for probable AD are selected.  Suitable patients
score in the 12-26 range on the Mini-Mental State Exam (MMSE).  Other selection
criteria are that patients are likely to survive the duration of the study and lack

complicating issues such as use of concomitant medications that may interfere. Baseline evaluations of patient function are made using classic psychometric measures, such as the MMSE, and the ADAS, which is a comprehensive scale for evaluating patients with Alzheimer's Disease status and function. These psychometric scales provide a measure

5      of progression of the Alzheimer's condition. Suitable qualitative life scales can also be used to monitor treatment. Disease progression can also be monitored by MRI. Blood profiles of patients can also be monitored including assays of immunogen-specific antibodies and T-cells responses.

        Following baseline measures, patients begin receiving treatment. They

10     are randomized and treated with either therapeutic agent or placebo in a blinded fashion. Patients are monitored at least every six months. Efficacy is determined by a significant reduction in progression of a treatment group relative to a placebo group.

        A second phase II trial is performed to evaluate conversion of patients from non-Alzheimer's Disease early memory loss, sometimes referred to as age-

15     associated memory impairment (AAMI) or mild cognitive impairment (MCI), to probable Alzheimer's disease as defined as by ADRDA criteria. Patients with high risk for conversion to Alzheimer's Disease are selected from a non-clinical population by screening reference populations for early signs of memory loss or other difficulties associated with pre-Alzheimer's symptomatology, a family history of Alzheimer's

20     Disease, genetic risk factors, age, sex, and other features found to predict high-risk for Alzheimer's Disease. Baseline scores on suitable metrics including the MMSE and the ADAS together with other metrics designed to evaluate a more normal population are collected. These patient populations are divided into suitable groups with placebo comparison against dosing alternatives with the agent. These patient populations are

25     followed at intervals of about six months, and the endpoint for each patient is whether or not he or she converts to probable Alzheimer's Disease as defined by ADRDA criteria at the end of the observation.


**General Materials and Methods**

30                    A. Preparation of Polyclonal and Monoclonal Aβ Antibodies

        The anti-Aβ polyclonal antibody was prepared from blood collected from two groups of animals. The first group consisted of 100 female Swiss Webster mice, 6

to 8 weeks of age. They were immunized on days 0, 15, and 29 with 100 μg of AN1792 combined with CFA/IFA. A fourth injection was given on day 36 with one-half the dose of AN1792. Animals were exsanguinated upon sacrifice at day 42, serum was prepared and the sera were pooled to create a total of 64 ml. The second group consisted of 24

5      female mice isogenic with the PDAPP mice but nontransgenic for the human APP gene, 6 to 9 weeks of age. They were immunized on days 0, 14, 28 and 56 with 100 μg of AN1792 combined with CFA/IFA. These animals were also exsanguinated upon sacrifice at day 63, serum was prepared and pooled for a total of 14 ml. The two lots of sera were pooled. The antibody fraction was purified using two sequential rounds of

10     precipitation with 50% saturated ammonium sulfate. The final precipitate was dialyzed against PBS and tested for endotoxin. The level of endotoxin was less than 1 EU/mg.

       The anti-Aβ monoclonal antibodies were prepared from ascites fluid. The fluid was first delipidated by the addition of concentrated sodium dextran sulfate to ice-cold ascites fluid by stirring on ice to a reach a final concentration of 0.238%.

15     Concentrated CaCl₂ was then added with stirring to reach a final concentration of 64mM. This solution was centrifuged at 10,000 x g and the pellet was discarded. The supernatant was stirred on ice with an equal volume of saturated ammonium sulfate added dropwise. The solution was centrifuged again at 10,000 x g and the supernatant was discarded. The pellet was resuspended and dialyzed against 20 mM Tris-HCl , 0.4

20     M NaCl, pH 7.5. This fraction was applied to a Pharmacia FPLC Sepharose Q Column and eluted with a reverse gradient from 0.4 M to 0.275 M NaCl in 20 mM Tris-HCl, pH 7.5.

       The antibody peak was identified by absorbance at 280 nm and appropriate fractions were pooled. The purified antibody preparation was characterized

25     by measuring the  protein concentration using the BCA method and the purity using SDS-PAGE. The pool was also tested for endotoxin. The level of endotoxin was less than 1 EU/mg. titers, titers less than 100 were arbitrarily assigned a titer value of 25.


       B.     Measurement of Antibody Titers

30     Mice were bled by making a small nick in the tail vein and collecting about 200 μl of blood into a microfuge tube. Guinea pigs were bled by first shaving the back hock area and then using an 18 gauge needle to nick the metatarsal vein and

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collecting the blood into microfuge tubes. Blood was allowed to clot for one hr at room temperature (RT), vortexed, then centrifuged at 14,000 x g for 10 min to separate the clot from the serum. Serum was then transferred to a clean microfuge tube and stored at 4°C until titered.

5          Antibody titers were measured by ELISA. 96-well microtiter plates (Costar EIA plates) were coated with 100 µl of a solution containing either 10 µg/ml either Aβ42 or SAPP or other antigens as noted in each of the individual reports in Well Coating Buffer (0.1 M sodium phosphate, pH 8.5, 0.1% sodium azide) and held overnight at RT. The wells were aspirated and sera were added to the wells starting at a

10        1/100 dilution in Specimen Diluent (0.014 M sodium phosphate, pH 7.4, 0.15 M NaCl, 0.6% bovine serum albumin, 0.05% thimerosal). Seven serial dilutions of the samples were made directly in the plates in three-fold steps to reach a final dilution of 1/218,700. The dilutions were incubated in the coated-plate wells for one hr at RT. The plates were then washed four times with PBS containing 0.05% Tween 20. The second antibody, a

15        goat anti-mouse Ig conjugated to horseradish peroxidase (obtained from Boehringer Mannheim), was added to the wells as 100 µl of a 1/3000 dilution in Specimen Diluent and incubated for one hr at RT. Plates were again washed four times in PBS, Tween 20. To develop the chromogen, 100 µl of Slow TMB (3,3',5,5'-tetramethyl benzidine obtained from Pierce Chemicals) was added to each well and incubated for 15 min at

20        RT. The reaction was stopped by the addition of 25 µl of 2 M $H_2SO_4$. The color intensity was then read on a Molecular Devices Vmax at (450 nm - 650 nm).

Titers were defined as the reciprocal of the dilution of serum giving one half the maximum OD. Maximal OD was generally taken from an initial 1/100 dilution, except in cases with very high titers, in which case a higher initial dilution was

25        necessary to establish the maximal OD. If the 50% point fell between two dilutions, a linear extrapolation was made to calculate the final titer. To calculate geometric mean antibody titers, titers less than 100 were arbitrarily assigned a titer value of 25.

### C.     Brain Tissue Preparation

30        After euthanasia, the brains were removed and one hemisphere was prepared for immunohistochemical analysis, while three brain regions (hippocampus, cortex and cerebellum) were dissected from the other hemisphere and used to measure

the concentration of various Aβ proteins and APP forms using specific ELISAs (Johnson-Wood *et al.*, *supra*).

   Tissues destined for ELISAs were homogenized in 10 volumes of ice-cold guanidine buffer (5.0 M guanidine-HCl, 50 mM Tris-HCl, pH 8.0). The

5   homogenates were mixed by gentle agitation using an Adams Nutator (Fisher) for three to four hr at RT, then stored at -20°C prior to quantitation of Aβ and APP. Previous experiments had shown that the analytes were stable under this storage condition, and that synthetic Aβ protein (Bachem) could be quantitatively recovered when spiked into homogenates of control brain tissue from mouse littermates (Johnson-Wood *et al.*,

10  *supra*).


### D.      Measurement of Aβ Levels

   The brain homogenates were diluted 1:10 with ice cold Casein Diluent (0.25% casein, PBS, 0.05% sodium azide, 20 μg/ml aprotinin, 5 mM EDTA pH 8.0, 10

15  μg/ml leupeptin) and then centrifuged at 16,000 x g for 20 min at 4° C. The synthetic Aβ protein standards (1-42 amino acids) and the APP standards were prepared to include 0.5 M guanidine and 0.1% bovine serum albumin (BSA) in the final composition. The "total" Aβ sandwich ELISA utilizes monoclonal antibody monoclonal antibody 266, specific for amino acids 13-28 of Aβ (Seubert *et al.*, *supra*), as the capture antibody, and

20  biotinylated monoclonal antibody 3D6, specific for amino acids 1-5 of Aβ (Johnson-Wood *et al.*, *supra*), as the reporter antibody. The 3D6 monoclonal antibody does not recognize secreted APP or full-length APP, but detects only Aβ species with an amino-terminal aspartic acid. This assay has a lower limit of sensitivity of ~50 ng/ml (11nM) and shows no cross-reactivity to the endogenous murine Aβ protein at concentrations up

25  to 1 ng/ml (Johnson-Wood *et al.*, *supra*).

   The Aβ1-42 specific sandwich ELISA employs mAβ 21F12, specific for amino acids 33-42 of Aβ (Johnson-Wood, *et al. supra*), as the capture antibody. Biotinylated mAβ 3D6 is also the reporter antibody in this assay which has a lower limit of sensitivity of about 125 μg/ml (28 μM, Johnson-Wood *et al.*, *supra*). For the Aβ

30  ELISAs, 100 μl of either mAβ 266 (at 10 μg/ml) or mAβ 21F12 at (5 μg/ml) was coated into the wells of 96-well immunoassay plates (Costar) by overnight incubation at

RT. The solution was removed by aspiration and the wells were blocked by the addition of 200 μl of 0.25% human serum albumin in PBS buffer for at least 1 hr at RT. Blocking solution was removed and the plates were stored desiccated at 4°C until used. The plates were rehydrated with Wash Buffer [Tris-buffered saline (0.15 M NaCl, 0.01

5    M Tris-HCl, pH 7.5), plus 0.05% Tween 20] prior to use. The samples and standards were added in triplicate aliquots of 100 μl per well and then incubated overnight at 4° C. The plates were washed at least three times with Wash Buffer between each step of the assay. The biotinylated mAβ 3D6, diluted to 0.5 μg/ml in Casein Assay Buffer (0.25% casein, PBS, 0.05% Tween 20, pH 7.4), was added and incubated in the wells for

10   1 hr at RT. An avidin-horseradish peroxidase conjugate, (Avidin-HRP obtained from Vector, Burlingame, CA), diluted 1:4000 in Casein Assay Buffer, was added to the wells for 1 hr at RT. The colorimetric substrate, Slow TMB-ELISA (Pierce), was added and allowed to react for 15 minutes at RT, after which the enzymatic reaction was stopped by the addition of 25 μl 2 N H2SO4. The reaction product was quantified using a

15   Molecular Devices Vmax measuring the difference in absorbance at 450 nm and 650 nm.


E.    Measurement of APP Levels

Two different APP assays were utilized. The first, designated APP-α/FL, 

20   recognizes both APP-alpha (α) and full-length (FL) forms of APP. The second assay is specific for APP-α. The APP-α /FL assay recognizes secreted APP including the first 12 amino acids of Aβ. Since the reporter antibody (2H3) is not specific to the α-clip-site, occurring between amino acids 612-613 of APP[695] (Esch *et al.*, Science 248:1122-1124 (1990)); this assay also recognizes full length APP (APP-FL). Preliminary

25   experiments using immobilized APP antibodies to the cytoplasmic tail of APP-FL to deplete brain homogenates of APP-FL suggest that approximately 30-40% of the APP-α /FL APP is FL (data not shown). The capture antibody for both the APP-α/FL and APP-α assays is mAb 8E5, raised against amino acids 444 to 592 of the APP[695] form (Games *et al.*, *supra*). The reporter mAb for the APP-α/FL assay is mAb 2H3, specific for

30   amino acids 597-608 of APP[695] (Johnson-Wood *et al.*, *supra*) and the reporter antibody for the APP-α assay is a biotinylated derivative of mAb 16H9, raised to amino acids 605

to 611 of APP. The lower limit of sensitivity of the APP-αFL assay is about 11 ng/ml (150 pM) (Johnson-Wood *et al.*) and that of the APP-α specific assay is 22 ng/ml (0.3 nM). For both APP assays, mAb 8E5 was coated onto the wells of 96-well EIA plates as described above for mAb 266. Purified, recombinant secreted APP-α was used as the

5 reference standard for the APP-α assay and the APP-α/FL assay (Esch *et al.*, *supra*). The brain homogenate samples in 5 M guanidine were diluted 1:10 in ELISA Specimen Diluent (0.014 M phosphate buffer, pH 7.4, 0.6% bovine serum albumin, 0.05% thimerosal, 0.5 M NaCl, 0.1% NP40). They were then diluted 1:4 in Specimen Diluent containing 0.5 M guanidine. Diluted homogenates were then centrifuged at 16,000 x g

10 for 15 seconds at RT. The APP standards and samples were added to the plate in duplicate aliquots and incubated for 1.5 hr at RT. The biotinylated reporter antibody 2H3 or 16H9 was incubated with samples for 1 hr at RT. Streptavidin-alkaline phosphatase (Boehringer Mannheim), diluted 1:1000 in specimen diluent, was incubated in the wells for 1 hr at RT. The fluorescent substrate 4-methyl-umbellipheryl-phosphate

15 was added for a 30-min RT incubation and the plates were read on a Cytofluor tm 2350 fluorimeter (Millipore) at 365 nm excitation and 450 nm emission.

F.     Immunohistochemistry

Brains were fixed for three days at 40C in 4% paraformaldehyde in PBS

20 and then stored from one to seven days at 4°C in 1% paraformaldehyde, PBS until sectioned. Forty-micron-thick coronal sections were cut on a vibratome at RT and stored in cryoprotectant (30% glycerol, 30% ethylene glycol in phosphate buffer) at -20°C prior to immunohistochemical processing. For each brain, six sections at the level of the dorsal hippocampus, each separated by consecutive 240 μm intervals, were

25 incubated overnight with one of the following antibodies: (1) a biotinylated anti-Aβ (mAb, 3D6, specific for human Aβ) diluted to a concentration of 2 μg/ml in PBS and 1% horse serum; or (2) a biotinylated mAb specific for human APP, 8E5, diluted to a concentration of 3 μg/ml in PBS and 1.0% horse serum; or (3) a mAb specific for glial fibrillary acidic protein (GFAP; Sigma Chemical Co.) diluted 1:500 with 0.25% Triton

30 X-100 and 1% horse serum, in Tris-buffered saline, pH 7.4 (TBS); or (4) a mAb specific for CD11b, MAC-1 antigen, (Chemicon International) diluted 1:100 with 0.25% Triton X-100 and 1% rabbit serum in TBS; or (5) a mAb specific for MHC II antigen,

(Pharmingen) diluted 1:100 with 0.25% Triton X-100 and 1% rabbit serum in TBS; or

(6) a rat mAb specific for CD 43 (Pharmingen) diluted 1:100 with 1% rabbit serum in

PBS or (7) a rat mAb specific for CD 45RA (Pharmingen) diluted 1:100 with 1% rabbit

serum in PBS; or (8) a rat monoclonal Aβ specific for CD 45RB (Pharmingen) diluted

5       1:100 with 1% rabbit serum in PBS; or (9) a rat monoclonal Aβ specific for CD 45

(Pharmingen) diluted 1:100 with 1% rabbit serum in PBS; or (10) a biotinylated

polyclonal hamster Aβ specific for CD3e (Pharmingen) diluted 1:100 with 1% rabbit

serum in PBS or (11) a rat mAb specific for CD3 (Serotec) diluted 1:200 with 1% rabbit

serum in PBS; or with (12) a solution of PBS lacking a primary antibody containing 1%

10      normal horse serum.

Sections reacted with antibody solutions listed in 1,2 and 6-12 above

were pretreated with 1.0% Triton X-100, 0.4% hydrogen peroxide in PBS for 20 min at

RT to block endogenous peroxidase. They were next incubated overnight at 4°C with

primary antibody. Sections reacted with 3D6 or 8E5 or CD3e mAbs were then reacted

15      for one hr at RT with a horseradish peroxidase-avidin-biotin-complex with kit

components "A" and "B" diluted 1:75 in PBS (Vector Elite Standard Kit, Vector Labs,

Burlingame, CA.). Sections reacted with antibodies specific for CD 45RA, CD 45RB,

CD 45, CD3 and the PBS solution devoid of primary antibody were incubated for 1 hour

at RT with biotinylated anti-rat IgG (Vector) diluted 1:75 in PBS or biotinylated anti-

20      mouse IgG (Vector) diluted 1:75 in PBS, respectively. Sections were then reacted for

one hr at RT with a horseradish peroxidase-avidin-biotin-complex with kit components

"A" and "B" diluted 1:75 in PBS (Vector Elite Standard Kit, Vector Labs, Burlingame,

CA.).

Sections were developed in 0.01% hydrogen peroxide, 0.05% 3,3'-

25      diaminobenzidine (DAB) at RT. Sections destined for incubation with the GFAP-,

MAC-1- AND MHC II-specific antibodies were pretreated with 0.6% hydrogen

peroxide at RT to block endogenous peroxidase then incubated overnight with the

primary antibody at 4°C. Sections reacted with the GFAP antibody were incubated for 1

hr at RT with biotinylated anti-mouse IgG made in horse (Vector Laboratories;

30      Vectastain Elite ABC Kit) diluted 1:200 with TBS. The sections were next reacted for

one hr with an avidin-biotin-peroxidase complex (Vector Laboratories; Vectastain Elite

ABC Kit) diluted 1:1000 with TBS. Sections incubated with the MAC-1-or MHC II-

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specific monoclonal antibody as the primary antibody were subsequently reacted for 1 hr

at RT with biotinylated anti-rat IgG made in rabbit diluted 1:200 with TBS, followed by

incubation for one hr with avidin-biotin-peroxidase complex diluted 1:1000 with TBS.

Sections incubated with GFAP-, MAC-1- and MHC II-specific antibodies were then

5      visualized by treatment at RT with 0.05% DAB, 0.01% hydrogen peroxide, 0.04%

nickel chloride, TBS for 4 and 11 min, respectively.

Immunolabeled sections were mounted on glass slides (VWR, Superfrost

slides), air dried overnight, dipped in Propar (Anatech) and overlaid with coverslips

using Permount (Fisher) as the mounting medium.

10     To counterstain Aβ plaques, a subset of the GFAP-positive sections were

mounted on Superfrost slides and incubated in aqueous 1% Thioflavin S (Sigma) for

7 min following immunohistochemical processing. Sections were then dehydrated and

cleared in Propar, then overlaid with coverslips mounted with Permount.


15     G.     Image Analysis

A Videometric 150 Image Analysis System (Oncor, Inc., Gaithersburg,

MD) linked to a Nikon Microphot-FX microscope through a CCD video camera and a

Sony Trinitron monitor was used for quantification of the immunoreactive slides. The

image of the section was stored in a video buffer and a color-and saturation-based

20     threshold was determined to select and calculate the total pixel area occupied by the

immunolabeled structures. For each section, the hippocampus was manually outlined

and the total pixel area occupied by the hippocampus was calculated. The percent

amyloid burden was measured as: (the fraction of the hippocampal area containing Aβ

deposits immunoreactive with mAb 3D6) x 100. Similarly, the percent neuritic burden

25     was measured as: (the fraction of the hippocampal area containing dystrophic neurites

reactive with monoclonal antibody 8E5) x100. The C-Imaging System (Compix, Inc.,

Cranberry Township, PA) operating the Simple 32 Software Application program was

linked to a Nikon Microphot-FX microscope through an Optronics camera and used to

quantitate the percentage of the retrospenial cortex occupied by GFAP-positive

30     astrocytes and MAC-1-and MHC II-positive microglia. The image of the

immunoreacted section was stored in a video buffer and a monochrome-based threshold

was determined to select and calculate the total pixel area occupied by immunolabeled

cells. For each section, the retrosplenial cortex (RSC) was manually outlined and the total pixel area occupied by the RSC was calculated. The percent astrocytosis was defined as: (the fraction of RSC occupied by GFAP-reactive astrocytes) X 100. Similarly, percent microgliosis was defined as: (the fraction of the RSC occupied by

5      MAC-1- or MHC II-reactive microglia) X 100. For all image analyses, six sections at the level of the dorsal hippocampus, each separated by consecutive 240 μm intervals, were quantitated for each animal. In all cases, the treatment status of the animals was unknown to the observer.


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       Although the foregoing invention has been described in detail for purposes of clarity of understanding, it will be obvious that certain modifications may be practiced within the scope of the appended claims. All publications and patent documents cited herein, as well as text appearing in the figures and sequence listing, are

15     hereby incorporated by reference in their entirety for all purposes to the same extent as if each were so individually denoted.


       From the foregoing it will be apparent that the invention provides for a number of uses. For example, the invention provides for the use of any of the antibodies

20     to Aβ described above in the treatment, prophylaxis or diagnosis of amyloidogenic disease, or in the manufacture of a medicament or diagnostic composition for use in the same.

We claim:

1.    A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 light chain variable region sequence, wherein the framework residue is selected from the group consisting of:

    (a)    a residue that non-covalently binds antigen directly;

    (b)    a residue adjacent to a CDR;

    (c)    a CDR-interacting residue; and

    (d)    a residue participating in the VL-VH interface.

2.    A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of:

    (a)    a residue that non-covalently binds antigen directly;

    (b)    a residue adjacent to a CDR;

    (c)    a CDR-interacting residue; and

    (d)    a residue participating in the VL-VH interface.

3.    The light chain of claim 1, wherein a CDR-interacting residue is identified by modeling the 3D6 light chain based on the solved structure of 1CR9.

4.    The light chain of claim 1, wherein a CDR-interacting residue is identified by modeling the 3D6 light chain based on the solved structure of 1NLD.

5.     The heavy chain of claim 2, wherein a CDR-interacting residue is identified by modeling the 3D6 heavy chain based on the solved structure of 1OPG.

6.     A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 light chain variable region sequence, wherein the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the 3D6 immunoglobulin light chain variable region.

7.     A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 3D6 heavy chain variable region sequence, wherein the framework residue is a residue capable of affecting heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the 3D6 immunoglobulin heavy chain variable region.

8.     The light chain of claim 6, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycoslyation site residue on the surface of the structural model.

9.      The heavy chain of claim 7, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, and a glycoslyation site residue on the surface of the structural model.


10.     The light chain of claim 6 or 8, wherein the framework residue is identified by modeling the 3D6 light chain based on the solved structure of 1CR9.


11.     The light chain of claim 6 or 8, wherein the frame work residue is identified by modeling the 3D6 light chain based on the solved structure of 1NLD.


12.     The heavy chain of claim 7 or 9, wherein the framework residue is identified by modeling the 3D6 heavy chain based on the solved structure of 1OPG.


13.     A humanized immunoglobulin light chain comprising (i) variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:2, and (ii) a variable framework region from a human acceptor immunoglobulin light chain, provided that at least one framework residue selected from the group consisting of L1, L2, L36 and L46 (Kabat numbering convention) is substituted with the corresponding amino acid residue from the mouse 3D6 light chain variable region sequence.


14.     A humanized immunoglobulin heavy chain comprising (i) variable region complementarity determining regions from the 3D6 heavy chain variable region sequence set forth as SEQ ID NO:4, and (ii) a variable framework regions from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue selected from the group consisting of H49, H93 and H94 (Kabat numbering convention) is substituted with the corresponding amino acid residue from the mouse 3D6 heavy chain variable region sequence.

15.     The light chain of any one claims 1, 3, 4, 6, 8, 10, 11 and 13, wherein the human acceptor light chain is of the subtype kappa II (Kabat convention).

16.     The heavy chain of any one claims 2, 5, 7, 9, 12 and 14, wherein the
5    human acceptor heavy chain is of the subtype III (Kabat convention).

17.     The light chain of claim 15, wherein the human acceptor light chain is selected from the group consisting of Kabat ID 019230, Kabat ID 005131, Kabat ID 005058, Kabat ID 005057, Kabat ID 005059, Kabat ID U21040 and Kabat ID U41645.
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18.     The light chain of claim 15, wherein the human acceptor light chain is Kabat ID 019230.

19.     The heavy chain of claim 16, wherein the human acceptor heavy chain is
15   selected from the group consisting of Kabat ID 045919, Kabat ID 000459, Kabat ID 000553, Kabat ID 000386 and Kabat ID M23691.

20.     The heavy chain of claim 16, wherein the human acceptor heavy chain is Kabat ID 045919.
20

21.     The light chain of any one of claims 1, 3, 4, 6, 8, 10, 11, 13, 15, 17 and 18, wherein at least one rare human framework residue is substituted with an amino acid residue which is common for human variable light chain sequences at that position.

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22.     The light chain of claim 1, 3, 4, 6, 8, 10, 11, 13, 15, 17 and 18,, wherein at least one rare human framework residue is substituted with a corresponding amino acid residue from a germline variable light chain sequence.

30       23.     The light chain of claim 22, wherein the germline variable light chain sequence is selected from the group consisting of A1, A17, A18, A2, and A19.

24.    The heavy chain of any one of claims 2, 5, 7, 9, 12, 14, 16, 19 and 20, wherein at least one rare human framework residue is substituted with an amino acid residue which is common for human variable heavy chain sequences at that position.

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25.    The heavy chain of any one of claims 2, 5, 7, 9, 12, 14, 16, 19 and 20, wherein at least one rare human framework residue is substituted with a corresponding amino acid residue from a germline variable heavy chain sequence.

10    26.    The heavy chain of claim 25, wherein the germline variable heavy chain sequence is selected from the group consisting of VH3-48, VH3-23, VH3-7, VH3-21 and VH3-11.

27.    The heavy chain of claim 25, wherein the germline variable heavy chain
15    sequence is VH3-23.

28.    The light chain of any one of claims 21-23, wherein the rare framework residue is selected based on occurrence at that position in less than 10% of human light chain variable region sequences in the light chain variable region subgroup, and the
20    common residue is selected based on an occurrence at that position in greater than 50% of sequences in the light chain variable region subgroup.

29.    The heavy chain of any one of claims 24-26, wherein the rare framework residue is selected based on occurrence at that position in less than 10% of human heavy
25    chain variable region sequences in the heavy chain variable region subgroup, and the common residue is selected based on an occurrence at that position in greater than 50% of sequences in the heavy chain variable region subgroup.

30.    A light chain comprising the complementarity determining regions
30    (CDRs) and variable region framework residues L1, L2, L36 and L46 (Kabat numbering convention) from the monoclonal antibody 3D6 light chain, wherein the remainder of the light chain is from a human immunoglobulin.

31.     A heavy chain comprising the complementarity determining regions (CDRs) and variable framework residues H49, H93 and H94 (Kabat numbering convention) from the monoclonal antibody 3D6 heavy chain, wherein the remainder of

5      the heavy chain is from a human immunoglobulin.

32.     A humanized immunoglobulin comprising the light chain of any one of claims 1, 3, 4, 6, 8, 10, 11, 13, 15, 17 and 18, and the heavy chain of any one of claims 2, 5, 7, 9, 12, 14, 16, 19 and 20, or antigen binding fragment of said immunoglobulin.

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33.     The immunoglobulin or antigen binding fragment of claim 32, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^7$ M$^{-1}$.

15     34.     The immunoglobulin or antigen binding fragment of claim 32, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^8$ M$^{-1}$.

35.     The immunoglobulin or antigen binding fragment of claim 32, which

20     specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^9$ M$^{-1}$.

36.     The immunoglobulin or antigen binding fragment of claim 32, wherein the heavy chain isotype is γ1.

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37.     The immunoglobulin or antigen binding fragment of claim 32, which binds to both soluble beta amyloid peptide (Aβ) and aggregated Aβ.

38.     The immunoglobulin of claim 37, wherein the soluble beta amyloid

30     peptide (Aβ) is disaggregated Aβ.

39.    The immunoglobulin or antigen binding fragment of claim 32, which mediates phagocytosis of beta amyloid peptide (Aβ).

40.    The immunoglobulin or antigen binding fragment of claim 32, which crosses the blood-brain barrier in a subject.

41.    The immunoglobulin or antigen binding fragment of claim 32, which reduces both beta amyloid peptide (Aβ) burden and neuritic dystrophy in a subject.

42.    A humanized immunoglobulin comprising a humanized heavy chain and a humanized light chain, wherein

(a)  the humanized light chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the mouse 3D6 immunoglobulin light chain variable domain designated SEQ ID NO:2, and a variable region framework from a human light chain variable region framework sequence provided that at least one position selected from a first group consisting of L1, L2, L36 and L46 (Kabat numbering convention) is occupied by the same amino acid residue present in the equivalent position of the mouse 3D6 immunoglobulin light chain variable region framework; and

(b)  the humanized heavy chain comprises three complementarity determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarity determining regions of the mouse 3D6 immunoglobulin heavy chain variable domain designated SEQ ID NO:4, and a variable region framework from a human heavy chain variable region framework sequence provided that at least one position selected from a second group consisting of H49, H93 and H94 (Kabat numbering convention) is occupied by the same amino acid residue present in the equivalent position of the mouse 3D6 immunoglobulin heavy chain variable region framework;

wherein the humanized immunoglobulin specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^7$ $M^{-1}$, wherein the 3D6 immunoglobulin has the light chain with a variable domain designated SEQ ID NO:2 and the heavy chain with a variable domain designated SEQ ID NO: 4.

43.     The humanized immunoglobulin of claim 42, wherein human light chain variable region framework is from a kappa light chain variable region.

44.     The humanized immunoglobulin of claim 42, wherein human heavy chain variable region framework is from an IgG1 heavy chain variable region.

45.     The humanized immunoglobulin of claim 42, wherein the humanized light chain variable region framework is from a light chain selected from the group consisting of Kabat ID 019230, Kabat ID 005131, Kabat ID 005058, Kabat ID 005057, Kabat ID 005059, Kabat ID U21040 and Kabat ID U41645.

46.     The humanized immunoglobulin of claim 42, wherein the humanized heavy chain variable region framework is from a heavy chain selected from the group consisting of Kabat ID 045919, Kabat ID 000459, Kabat ID 000553, Kabat ID 000386 and Kabat ID M23691.

47.     The humanized immunoglobulin of claim 42, wherein the humanized light chain variable region framework is identical to the Kabat ID 019230 light chain variable region framework sequence except for the positions from the first group, and the heavy chain variable region framework is identical to the Kabat ID 045919 heavy chain variable region framework sequence except for the positions from the second group.

48. The humanized immunoglobulin of claim 42, wherein the humanized light chain comprises complementarity determining regions that are identical to the corresponding complementarity determining regions of the mouse 3D6 heavy chain, and the humanized heavy chain comprises complementarity determining regions that are

identical to the corresponding complementarity determining regions of the mouse 3D6 heavy chain.

49.     A humanized antibody comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 3D6 variable light chain sequence set forth as SEQ ID NO:2.

50.     A humanized antibody comprising the complementarity determining regions (CDR1, CDR2 and CDR3) of the 3D6 variable heavy chain sequence set forth as SEQ ID NO:4.

51.     A humanized antibody, or antigen-binding fragment thereof, which specifically binds to beta amyloid peptide (Aβ), comprising a variable region comprising complementarity determining regions (CDRs) corresponding to CDRs from the mouse 3D6 antibody.

52.     A humanized antibody which binds beta amyloid peptide (Aβ) with an affinity of at least $10^7$ $M^{-1}$ comprising:

(a) a light chain variable domain comprising murine 3D6 complementarity determining region (CDR) amino acid residues and human VL subgroup II variable domain framework region (FR) amino acid residues; and

(b) a heavy chain variable domain comprising murine 3D6 complementarity determining region (CDR) amino acid residues and human VH subgroup III variable domain framework region (FR) amino acid residues.

53.     A chimeric immunoglobulin comprising variable region complementarity determining regions (CDRs) from the 3D6 immunoglobulin variable region sequences set forth as SEQ ID NO:2 or SEQ ID NO:4, and variable framework regions from a human acceptor immunoglobulin.

54.     An immunoglobulin, or antigen-binding fragment thereof, comprising a variable heavy chain region as set forth in SEQ ID NO:8 and a variable light chain region as set forth in SEQ ID NO:5.

5       55.     An immunoglobulin, or antigen-binding fragment thereof, comprising a variable heavy chain region as set forth in SEQ ID NO:12 and a light chain region as set forth in SEQ ID NO:11.

56.     An immunoglobulin comprising a variable heavy chain region as set forth
10   in SEQ ID NO:8, a variable light chain region as set forth in SEQ ID NO:5, and constant regions from IgG1.

57.     An immunoglobulin comprising a variable heavy chain region as set forth in SEQ ID NO:12, a light chain region as set forth in SEQ ID NO:11, and constant
15   regions from IgG1.

58.     A method of preventing or treating an amyloidogenic disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 32-52.
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59.     A method of preventing or treating Alzheimer's disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 32-52.

25   60.     The method of claim 59, wherein the effective dosage of humanized immunoglobulin is 1 mg/kg body weight.

61.     The method of claim 59, wherein the effective dosage of humanized immunoglobulin is 10 mg/kg body weight.
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62.     A pharmaceutical composition comprising the immunoglobulin of any one of claims 32-52 and a pharmaceutical carrier.

63.    An isolated polypeptide comprising a fragment of SEQ ID NO:2 selected from the group consisting of amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

64.    An isolated polypeptide comprising amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

65.    An isolated polypeptide comprising a fragment of SEQ ID NO:4 selected from the group consisting of amino acids 31-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-107 of SEQ ID NO:4.

66.    An isolated polypeptide comprising amino acids 31-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-107 of SEQ ID NO:4.

67.    An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:2.

68.    An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:4.

69.    A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:2, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to direct specific binding to beta amyloid peptide (Aβ) with a binding affinity of at least $10^7$ M$^{-1}$.

70.    A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:4, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to specifically bind beta amyloid peptide (Aβ) with a binding affinity of at least $10^7$ M$^{-1}$.

71.     An isolated polypeptide comprising residues 1-112 of the amino acid sequence of SEQ ID NO:2 or comprising residues 1-119 of the amino acid sequence of SEQ ID NO:4.

5          72.     An isolated nucleic acid molecule encoding the light chain of any one of claims 1, 3, 4, 6, 8, 10, , 11, 13, 15, 17 and 18.

73.     An isolated nucleic acid molecule encoding the heavy chain of any one of claims 2, 5, 7, 9, 12, 14, 16, 19 and 20.

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74.     An isolated nucleic acid molecule encoding the polypeptide of any one of claims 64-71.

75.     An isolated nucleic acid molecule encoding the immunoglobulin of any
15     one of claims 32-57.

76.     An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:1 or 3.

20          77.     A vector comprising the nucleic acid molecule of any of claims 72-76.

78.     A host cell comprising the nucleic acid molecule of any of claims 72-76.

79.     A method of producing an antibody, or fragment thereof, comprising
25     culturing the host cell of claim 45 under conditions such that the antibody or fragment is produced and isolating said antibody from the host cell or culture.

80.     A method of producing an antibody or fragment thereof, said method comprising culturing a host cell that expresses a nucleic acid molecule encoding said
30     antibody or fragment under conditions such that the antibody or fragment is produced, and isolating said antibody or fragment from the host cell or culture, wherein said

antibody or fragment comprises amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

81.     A method of producing an antibody or fragment thereof, said method
5   comprising culturing a host cell that expresses a nucleic acid molecule encoding said antibody or fragment, under conditions such that the antibody or fragment is produced, and isolating said antibody from the host cell or culture, wherein said antibody or fragment comprises amino acids 31-35 of SEQ ID NO:4, amino acids 50-66 of SEQ ID NO:4 and amino acids 99-112 of SEQ ID NO:4.
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82.     A method for identifying residues amenable to substitution in a humanized 3D6 immunoglobulin variable framework region, comprising modeling the three-dimensional structure of the 3D6 variable region based on a solved immunoglobulin structure and analyzing said model for residues capable of affecting
15   3D6 immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified.

83.     Use of the variable region sequence set forth as SEQ ID NO:2 or SEQ ID NO:4, or any portion thereof, in producing a three-dimensional image of a 3D6
20   immunoglobulin, 3D6 immunoglobulin chain, or domain thereof.

84.     A humanized immunoglobulin light chain comprising (i) variable region complementary determining regions (CDRs) from the 10D5 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:14, and (ii) a variable framework
25   region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 10D5 light chain variable region sequence, wherein the framework residue is selected from the group consisting of:
        (a)     a residue that non-covalently binds antigen directly;
30      (b)     a residue adjacent to a CDR;
        (c)     a CDR-interacting residue; and
        (d)     a residue participating in the VL-VH interface.

85.    A humanized immunoglobulin heavy chain comprising (i) variable region complementary determining regions (CDRs) from the 10D5 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:16, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 10D5 heavy chain variable region sequence, wherein the framework residue is selected from the group consisting of:

    (a)    a residue that non-covalently binds antigen directly;

    (b)    a residue adjacent to a CDR;

    (c)    a CDR-interacting residue; and

    (d)    a residue participating in the VL-VH interface.


86.    The light chain of claim 84, wherein a CDR-interacting residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 70% sequence identity with the 10D5 light chain.


87.    The light chain of claim 84, wherein a CDR-interacting residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 80% sequence identity with the 10D5 light chain.


88.    The light chain of claim 84, wherein a CDR-interacting residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 90% sequence identity with the 10D5 light chain.


89.    The heavy chain of claim 85, wherein a CDR-interacting residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chan that shares at least 70% sequence identity with the 10D5 heavy chain.

90.    The heavy chain of claim 85, wherein a CDR-interacting residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chan that shares at least 80% sequence identity with the 10D5 heavy chain.

91.    The heavy chain of claim 85, wherein a CDR-interacting residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chan that shares at least 90% sequence identity with the 10D5 heavy chain.

92.    A humanized immunoglobulin light chain comprising (i) variable region complementary determining regions (CDRs) from the 10D5 immunoglobulin light chain variable region sequence set forth as SEQ ID NO:14, and (ii) a variable framework region from a human acceptor immunoglobulin light chain sequence, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 10D5 light chain variable region sequence, wherein the framework residue is a residue capable of affecting light chain variable region conformation or function as identified by analysis of a three-dimensional model of the 10D5 immunoglobulin light chain variable region.

93.    A humanized immunoglobulin heavy chain comprising (i) variable region complementary determining regions (CDRs) from the 10D5 immunoglobulin heavy chain variable region sequence set forth as SEQ ID NO:16, and (ii) a variable framework region from a human acceptor immunoglobulin heavy chain, provided that at least one framework residue is substituted with the corresponding amino acid residue from the mouse 10D5 heavy chain variable region sequence, wherein the framework residue is a residue capable of affecting heavy chain variable region conformation or function as identified by analysis of a three-dimensional model of the 10D5 immunoglobulin heavy chain variable region.

94.    The light chain of claim 92, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, a rare residue, and a glycoslyation site residue on the surface of the structural model.

95.    The heavy chain of claim 93, wherein the framework residue is selected from the group consisting of a residue capable of interacting with antigen, a residue proximal to the antigen binding site, a residue capable of interacting with a CDR, a residue adjacent to a CDR, a residue within 6 Å of a CDR residue, a canonical residue, a vernier zone residue, an interchain packing residue, an unusual residue, and a glycoslyation site residue on the surface of the structural model.

96.    The light chain of claim 92 or 94, wherein the framework residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 70% sequence identity with the 10D5 light chain.

97.    The light chain of claim 92 or 94, wherein the framework residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 80% sequence identity with the 10D5 light chain.

98.    The light chain of claim 92 or 94, wherein the framework residue is identified by modeling the 10D5 light chain based on the solved structure of a murine immunoglobulin light chain that shares at least 90% sequence identity with the 10D5 light chain.

99.     The heavy chain of claim 93 or 95, wherein the framework residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 70% sequence identity with the 10D5 heavy chain.

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100.    The heavy chain of claim 93 or 95, wherein the framework residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 80% sequence identity with the 10D5 heavy chain.

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101.    The heavy chain of claim 93 or 95, wherein the framework residue is identified by modeling the 10D5 heavy chain based on the solved structure of a murine immunoglobulin heavy chain that shares at least 90% sequence identity with the 10D5 heavy chain..

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102.    The light chain of any one of claims 84, 86-88, 92, 94 and 96-98, wherein at least one rare human framework residue is substituted with an amino acid residue which is common for human variable light chain sequences at that position.

20      103.    The light chain of any one of claims 84, 86-88, 92, 94 and 96-98, wherein at least one rare human framework residue is substituted with a corresponding amino acid residue from a germline variable light chain sequence.

104.    The light chain of claim 103, wherein the germline variable light chain

25  sequence is that of an immunoglobulin sharing at least 70% identity with the variable light chain sequence of sequence identity.

105.    The light chain of claim 103, wherein the germline variable light chain sequence is that of an immunoglobulin sharing at least 80% identity with the variable

30  light chain sequence of sequence identity.

106.    The light chain of claim 103, wherein the germline variable light chain sequence is that of an immunoglobulin sharing at least 90% identity with the variable light chain sequence of sequence identity.

5       107.    The heavy chain of any one of claims 85, 89-91, 93 and 99-101, wherein at least one rare human framework residue is substituted with an amino acid residue which is common for human variable heavy chain sequences at that position.

108.    The heavy chain of any one of claims 85, 89-91, 93 and 99-101, wherein

10      at least one rare human framework residue is substituted with a corresponding amino acid residue from a germline variable heavy chain sequence.

109.    The heavy chain of claim 108, wherein the germline variable heavy chain sequence is that of or is sharing of at least 70% identity with the variable heavy chain

15      sequence of SEQ ID NO:16.

110.    The heavy chain of claim 108, wherein the germline variable heavy chain sequence is that of or is sharing of at least 80% identity with the variable heavy chain sequence of sequence identity no. 16.

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111.    The heavy chain of claim 108, wherein the germline variable heavy chain sequence is that of or is sharing of at least 90% identity with the variable heavy chain sequence of sequence identity no. 16.

25      112.    The light chain of any one of claims 102-106, wherein the rare framework residue is selected based on occurrence at that position in less than 10% of human light chain variable region sequences in the light chain variable region subgroup, and the common residue is selected based on an occurrence at that position in greater than 50% of sequences in the light chain variable region subgroup.

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113.    The heavy chain of any one of claims 107-111, wherein the rare framework residue is selected based on occurrence at that position in less than 10% of human heavy chain variable region sequences in the heavy chain variable region subgroup, and the common residue is selected based on an occurrence at that position in greater than 50% of sequences in the heavy chain variable region subgroup.

114.    A humanized immunoglobulin comprising the light chain of any one of claims 84, 86-88, 92, 94 and 96-98, and the heavy chain of any one of claims 85, 89-91, and 93 and 99-101, or antigen-binding fragment of said immunoglobulin.

115.    The immunoglobulin or antigen binding fragment of claim 114, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^{-7}$ M.

116.    The immunoglobulin or antigen binding fragment of claim 114, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^{-8}$ M.

117.    The immunoglobulin or antigen binding fragment of claim 114, which specifically binds to beta amyloid peptide (Aβ) with a binding affinity of at least $10^{-9}$ M.

118.    The immunoglobulin or antigen binding fragment of claim 114, wherein the heavy chain isotype is γ1.

119.    The immunoglobulin or antigen binding fragment of claim 114, which binds to aggregated beta amyloid peptide (Aβ).

120.    The immunoglobulin or antigen binding fragment of claim 114, which mediates phagocytosis of beta amyloid peptide (Aβ).

121.    The immunoglobulin or antigen binding fragment of claim 114, which crosses the blood-brain barrier in a subject.

122.    The immunoglobulin or antigen binding fragment of claim 114, which reduces beta amyloid peptide (Aβ) plaque burden in a subject.

123.    A humanized immunoglobulin comprising a humanized heavy chain and a humanized light chain, wherein

(a) the humanized light chain comprises three complementary determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementarily determining regions of the mouse 10D5 immunoglobulin light chain variable domain designated SEQ ID NO:14, and a variable region framework from a human light chain variable region framework sequence provided that at least one framework residue selected from the group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue, is occupied by the same amino acid residue present in the equivalent position of the mouse 10D5 immunoglobulin light chain variable region framework; and

(b) the humanized heavy chain comprises three complementary determining regions (CDR1, CDR2 and CDR3) having amino acid sequences from the corresponding complementary determining regions of the mouse 10D5 immunoglobulin heavy chain variable domain designated SEQ ID NO:16, and a variable region framework from a human heavy chain variable region framework sequence provided that at least one framework residue selected from a second group consisting of a canonical residue, a vernier residue, a packing residue and a rare residue, is occupied by the same amino acid residue present in the equivalent position of the mouse 10D5 immunoglobulin heavy chain variable region framework;

wherein the humanized immunoglobulin specifically binds to beta amyloid peptide ("Aβ") with a binding affinity of at least $10^{-7}$ M, wherein the 10D5 immunoglobulin has the light chain with a variable domain designated SEQ ID NO:14 and the heavy chain with a variable domain designated SEQ ID NO:16.

124.    The humanized immunoglobulin of claim 123, wherein human light chain variable region framework is from a kappa light chain variable region.

125.    The humanized immunoglobulin of claim 123, wherein human heavy chain variable region framework is from an IgG1 heavy chain variable region.

126.    The humanized immunoglobulin of claim 123, wherein the light chain variable region framework is from a human immunoglobulin light chain having at least 70% sequence identity with light chain sequence of the 10D5 immunoglobulin.

127.    The humanized immunoglobulin of claim 123, wherein the heavy chain variable region framework is from a human immunoglobulin heavy chain having at least 70% sequence identity with heavy chain sequence of the 10D5 immunoglobulin..

128.    The humanized immunoglobulin of claim 123, wherein the humanized light chain comprises complementary determining regions that are identical to the corresponding complementary determining regions of the mouse 10D5 heavy chain, and the humanized heavy chain comprises complementary determining regions that are identical to the corresponding complementary determining regions of the mouse 10D5 heavy chain.

129.    A humanized antibody comprising the complementary determining regions (CDR1, CDR2 and CDR3) of the 10D5 variable light chain sequence set forth as SEQ ID NO:14.

130.    A humanized antibody comprising the complementary determining regions (CDR1, CDR2 and CDR3) of the 10D5 variable heavy chain sequence set forth as SEQ ID NO:16.

131.    A humanized antibody, or antigen-binding fragment thereof, which specifically binds to beta amyloid peptide (Aβ), comprising a variable region comprising complementary determining regions (CDRs) corresponding to CDRs from the mouse 10D5 antibody.

5

132.    A chimeric immunoglobulin comprising variable region sequence substantially as set forth in SEQ ID NO:14 or SEQ ID NO:16, and constant region sequences from a human immunoglobulin.

10      133.    A method of preventing or treating an amyloidogenic disease in a patient, comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 114-131.

134.    A method of preventing or treating Alzheimer's disease in a patient,
15      comprising administering to the patient an effective dosage of the humanized immunoglobulin of any one of claims 114-131.

135.    The method of claim 134, wherein the effective dosage of humanized immunoglobulin is 1 mg/kg body weight.

20

136.    The method of claim 134, wherein the effective dosage of humanized immunoglobulin is 10 mg/kg body weight.

137.    A pharmaceutical composition comprising the immunoglobulin of any
25      one of claims 114-131 and a pharmaceutical carrier.

138.    An isolated polypeptide comprising a fragment of SEQ ID NO:2 selected from the group consisting of amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

30

139.    An isolated polypeptide comprising amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

140. An isolated polypeptide comprising a fragment of SEQ ID NO:4 selected from the group consisting of amino acids 31-37 of SEQ ID NO:4, amino acids 52-67 of SEQ ID NO:4 and amino acids 100-112 of SEQ ID NO:4.

141. An isolated polypeptide comprising amino acids 31-37 of SEQ ID NO:4, amino acids 52-67 of SEQ ID NO:4 and amino acids 100-112 of SEQ ID NO:4.

142. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:14.

143. An isolated polypeptide comprising the amino acid sequence of SEQ ID NO:16.

144. A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:14, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to specifically bind beta amyloid peptide (Aβ) with a binding affinity of at least $10^{-7}$ M.

145. A variant of a polypeptide comprising the amino acid sequence of SEQ ID NO:16, said variant comprising at least one conservative amino acid substitution, wherein the variant retains the ability to direct specific binding to beta amyloid peptide (Aβ) with a binding affinity of at least $10^{-7}$ M.

146. An isolated polypeptide comprising residues 1-112 of the amino acid sequence of SEQ ID NO:14 or comprising residues 1-123 of the amino acid sequence of SEQ ID NO:16.

147. An isolated nucleic acid molecule encoding the light chain of any one of claims 84, 86-88, 92, 94 and 96-98.

148.    An isolated nucleic acid molecule encoding the heavy chain of any one of claims 85, 89-91, 93 and 99-101.

149.    An isolated nucleic acid molecule encoding the polypeptide of any one of claims 139-146.

150.    An isolated nucleic acid molecule encoding the immunoglobulin of any one of claims 114-132.

151.    An isolated nucleic acid molecule comprising the nucleotide sequence of SEQ ID NO:13 or 15.

152.    A vector comprising the nucleic acid molecule of any of claims 147-151.

153.    A host cell comprising the nucleic acid molecule of any of claims 147-151.

154.    A method of producing an antibody, or fragment thereof, comprising culturing the host cell of claim 153 under conditions such that the antibody or fragment is produced and isolating said antibody from the host cell or culture.

155.    A method of producing an antibody, or fragment thereof, said method comprising culturing a host cell that expresses a nucleic acid molecule encoding said antibody or fragment under conditions such that the antibody or fragment is produced, and isolating said antibody from the host cell or culture, wherein said antibody or fragment comprises amino acids 24-39 of SEQ ID NO:2, amino acids 55-61 of SEQ ID NO:2 and amino acids 94-102 of SEQ ID NO:2.

156.    A method of producing an antibody, or fragment thereof, said method comprising culturing a host cell that expresses a nucleic acid molecule encoding said antibody or fragment under conditions such that the antibody or fragment is produced, and isolating said antibody from the host cell or culture, wherein said antibody or

fragment comprises amino acids 31-37 of SEQ ID NO:4, amino acids 52-67 of SEQ ID NO:4 and amino acids 100-112 of SEQ ID NO:4.

5      157.    A method for identifying residues amenable to substitution in a humanized 10D5 immunoglobulin variable framework region, comprising modeling the three-dimensional structure of the 10D5 variable region based on a solved immunoglobulin structure and analyzing said model for residues capable of affecting 10D5 immunoglobulin variable region conformation or function, such that residues amenable to substitution are identified.

10

158.    Use of the variable region sequence set forth as SEQ ID NO:14 or SEQ ID NO:16, or any portion thereof, in producing a three-dimensional image of a 10D5 immunoglobulin, 10D5 immunoglobulin chain, or domain thereof.

# FIG. 1
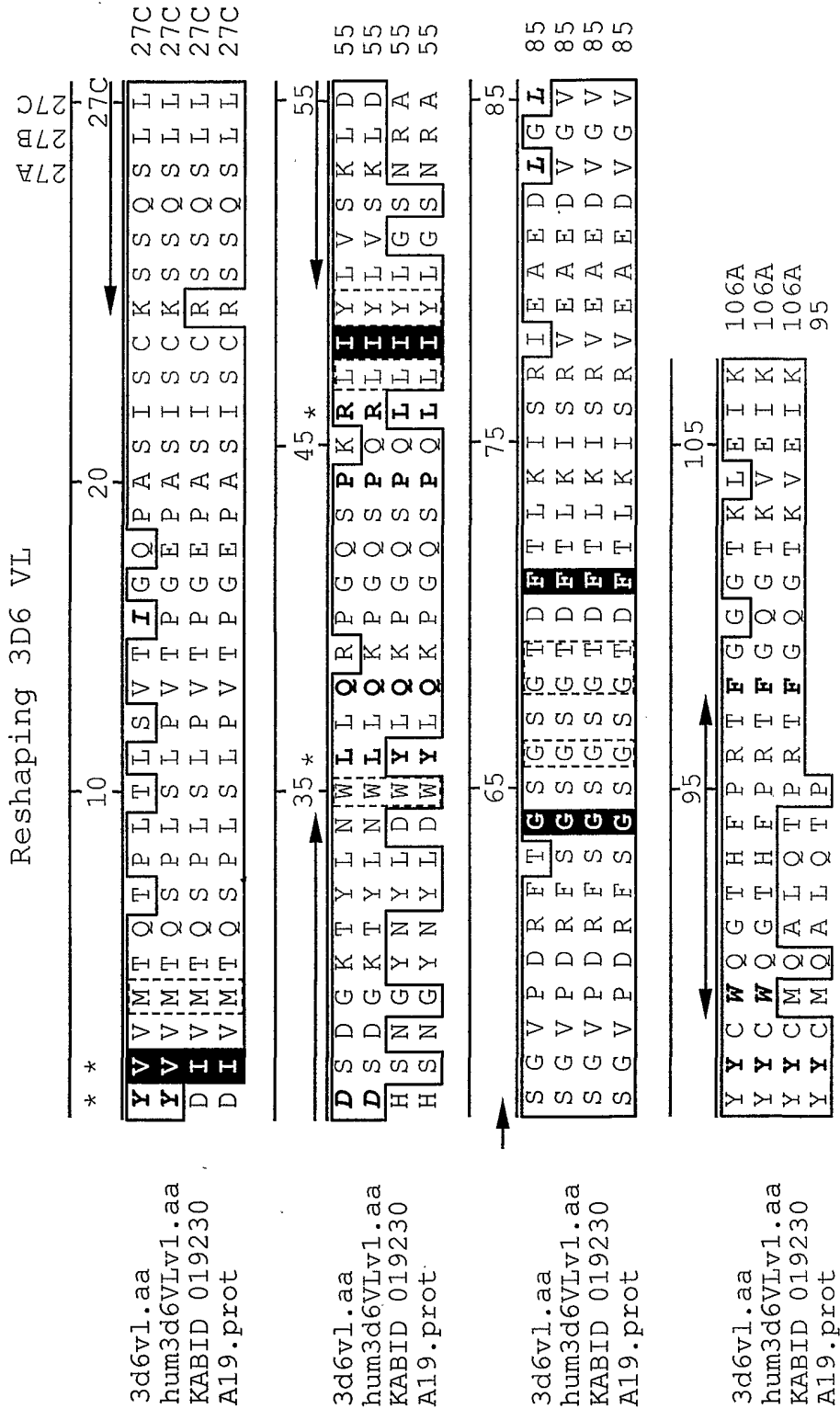
Reshaping 3D6 VL



'Decoration #1': Box residues that match hum3D6VLv1.aa exactly. Residue numbering cf Kabat
3d6v1.aa - Donor murine sequence          hum3d6VLv1.aa - humanized 3d6
KABID 019230 - human acceptor framework   A19.prot - human germline VH

# FIG. 2

Reshaping 3D6 VH
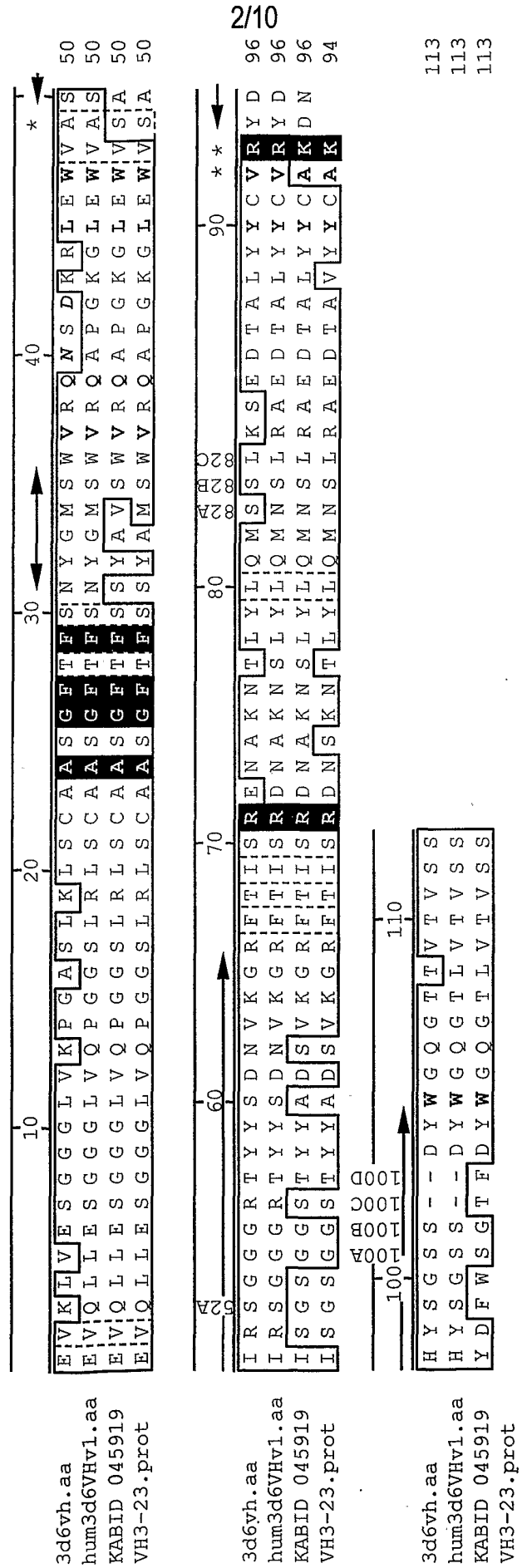


'Decoration #1': Box residues that match hum3d6VHv1.aa exactly.  Residue Numbering cf Kabat

3d6vh.aa – Donor murine sequence
hum3d6VHv1.aa – humanized 3d6 VH
KABID 045919 – human acceptor framework
VH3-23.prot – human germline VH

# FIG. 3A

Aβ42 ELISA



—□— 3D6 (αMs-HRP)

—○— PK1614
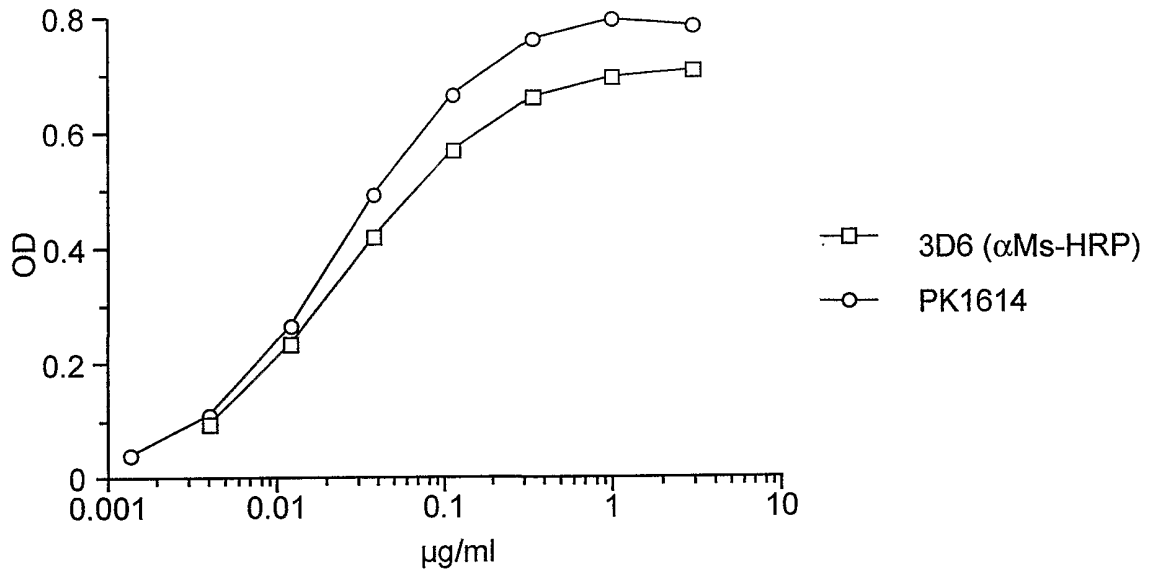
# FIG. 3B

Aβ42 ELISA
competition of 3D6-B



—□— 3D6

—○— PK1614

—⊞— 10D5
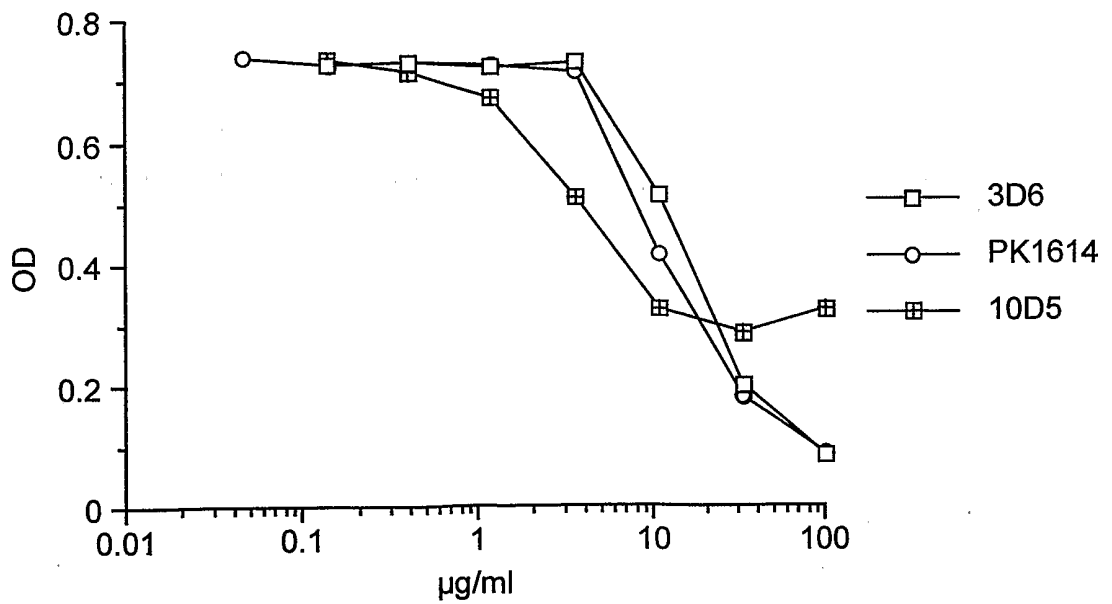
FIG. 4
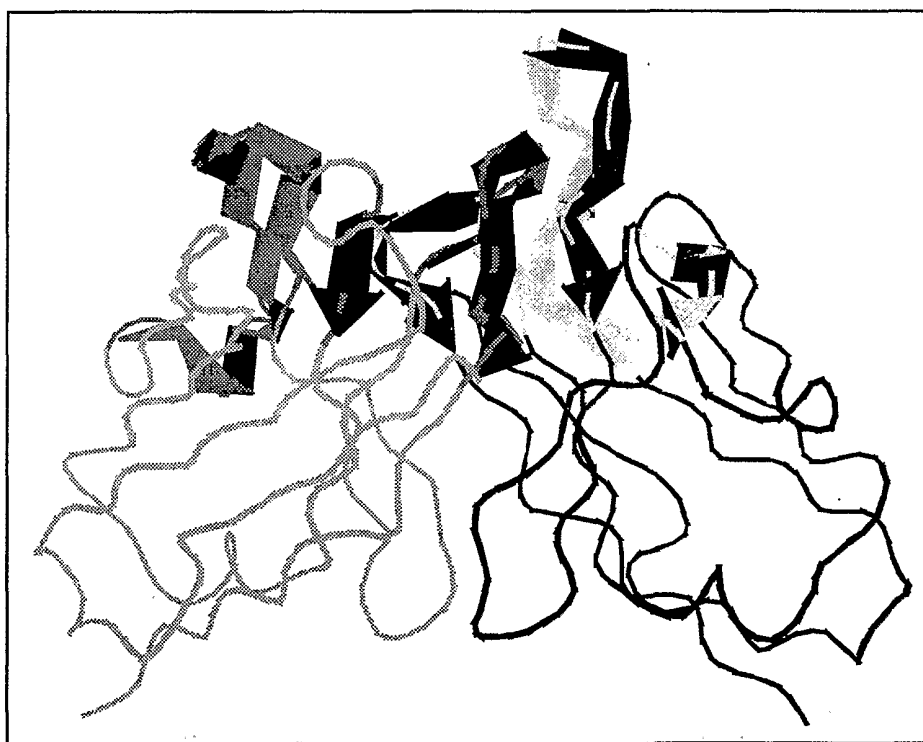
# FIG. 5B

MF011101 Agg. ABeta ELISA



□ lot 840 H1L2
△ lot 837 H1L1
▽ lot 842 H1L1
◇ lot 843 H2L2
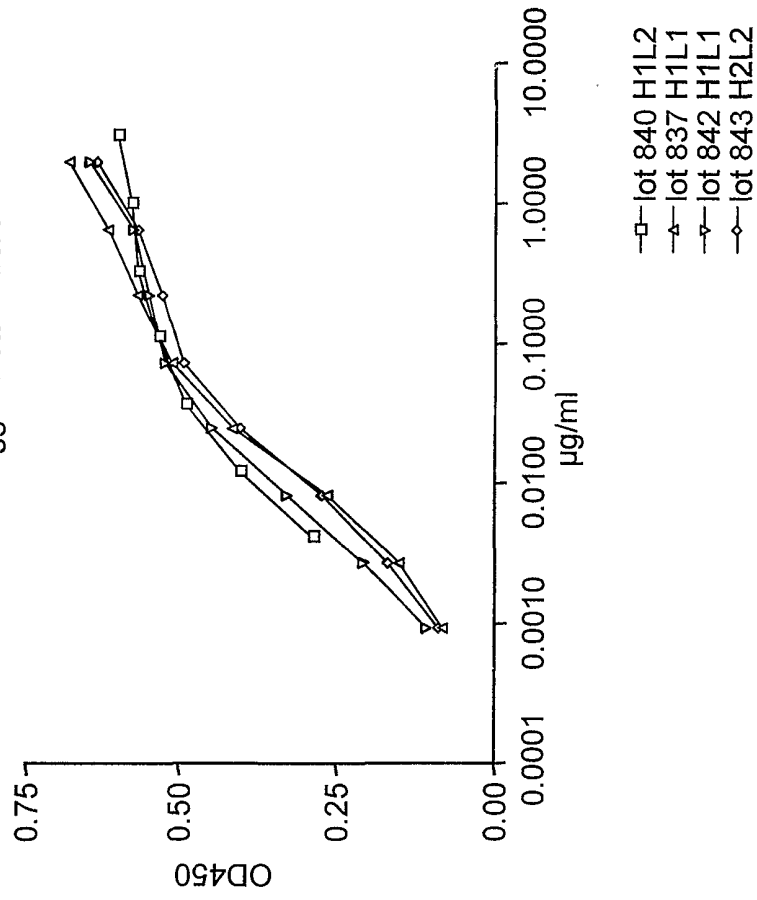
# FIG. 5A

MF121400 Agg. ABeta ELISA



□ h3D6 v1 lot#837
○ h3D6 v1 lot#837b
▽ ch3D6 PK1614
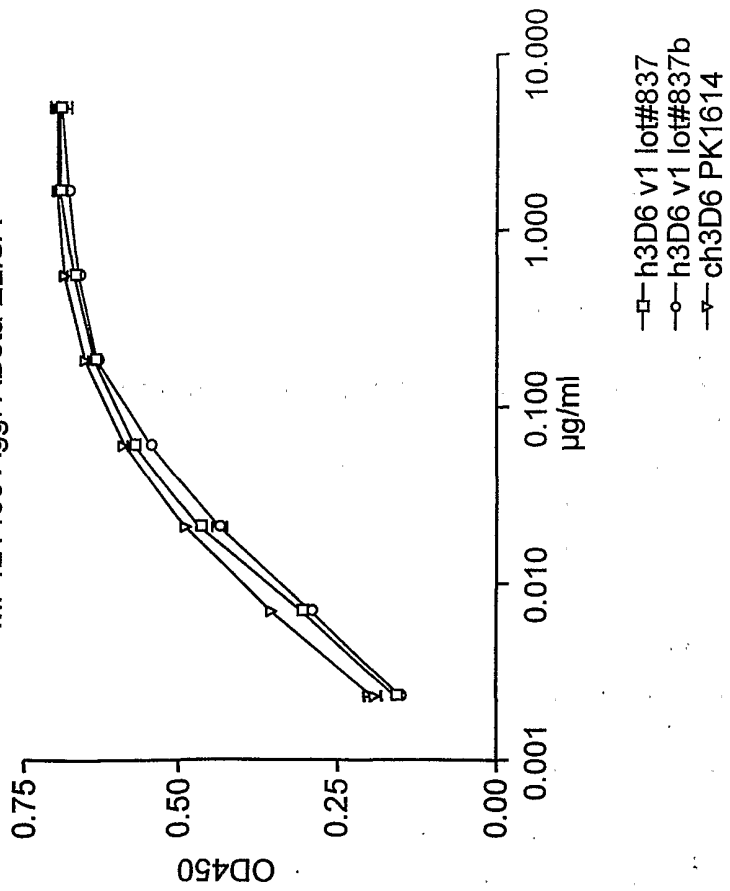
# FIG. 6

Staining of PDAPP brain sections with humanized 3D6

MAb Titrations on PD-APP Brain Sections
Channels 70-256

FIG. 7

h3D6 affinity: Competitive Binding
Competition on Aβ42

3D6
PK1614 chimeric
PK1636 V1
H2L2
10D5
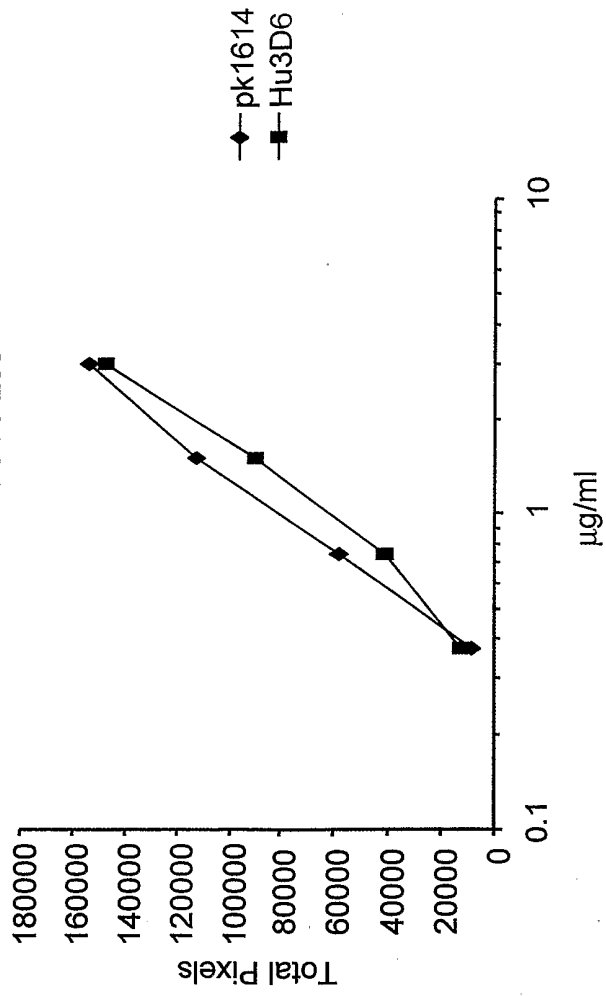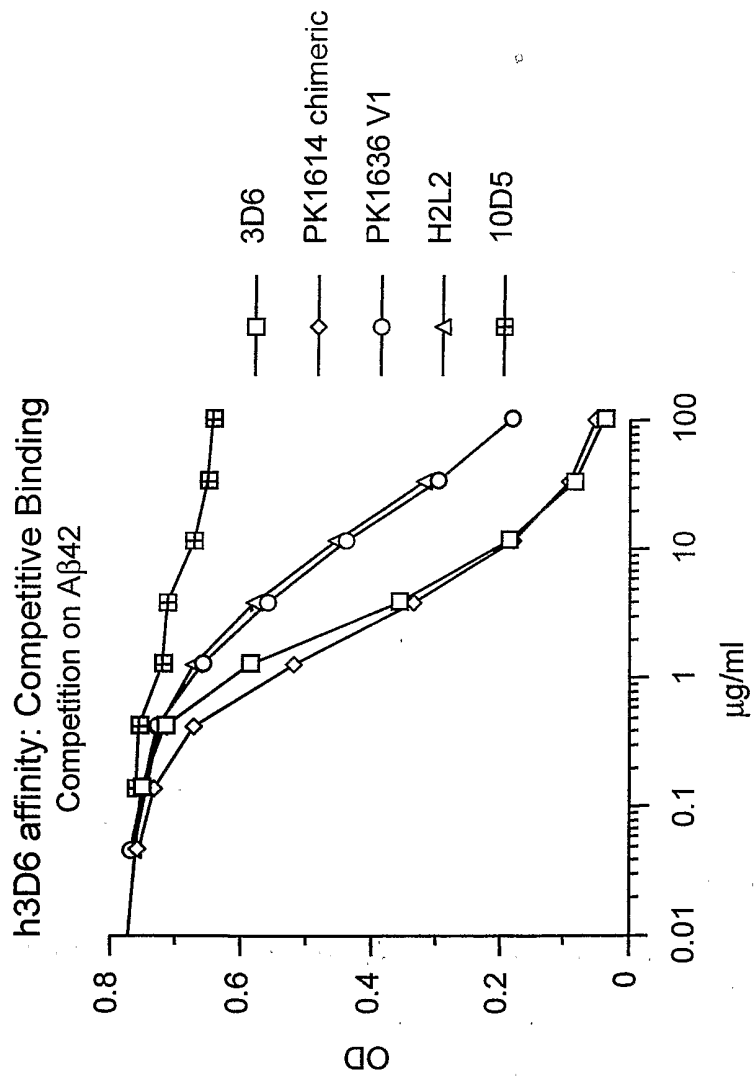
FIG. 8

Ex-vivo assay: Stimulation of microglial phagocytosis by h3D6
Ex vivo PDAPP
266/3D6-B
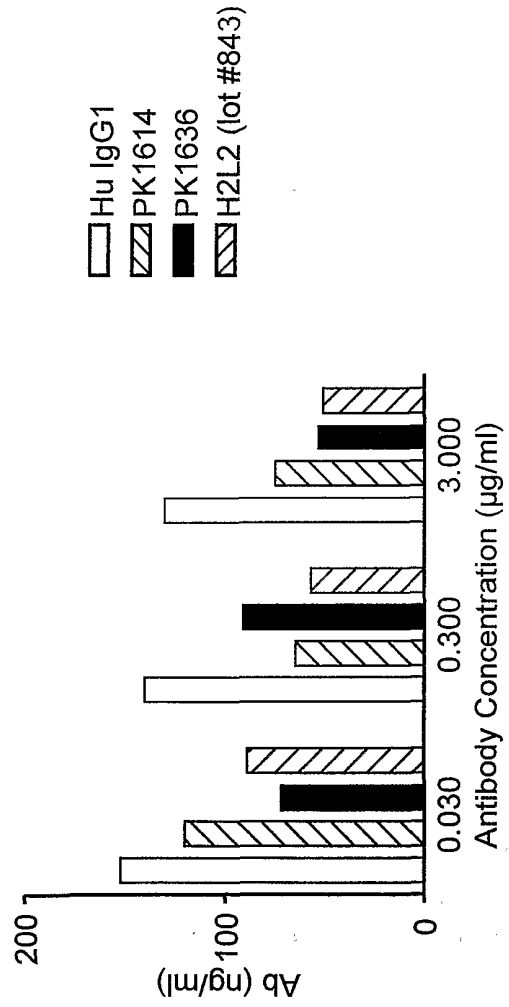
# FIG. 9

```
                    10        20          29
                    |         |    |      |30
10D5vl.pro   MKLPVRLL-VLMFWIPASSSDVLMTQTPLS
 3D6vl.pro   MMSPAQFLFLLVLWIRETNGYVVMTQTPLT

             30      40        50         59
             |       |         |          |60
10D5vl.pro   LPVSLGDQASISCRSSQNIHSNGNTYLEW
 3D6vl.pro   LSVTIHPASQSISCKSSQSILDSDGKTYLNW

             60      70        80         89
             |       |         |          |90
10D5vl.pro   YLQKPGQSPKLLIHYKVSNRFSGVPDRFSGS
 3D6vl.pro   LLQRPGQSPRLLIYLVSKLDSGVPDRFTGS

             90     100       110        119
             |      |         |          |120
10D5vl.pro   GSGTDFTLKIHKVEAEDLGIYYCFQGSHVP
 3D6vl.pro   GSGTDFTLKISRIEAEDLGLYYCWQGTHFP

             120    130       131
             |      |         |132
10D5vl.pro   LTFGAGTKLELE
 3D6vl.pro   RTFGGGTKLEIK
```

# FIG. 10

```
                10        20        30
                |         |         |
10D5vh.pro  MD-RLTSSFLLLLIVPAYVLSQATLKESGPG          29
3D6vh.PRO   MNFGLSLIFLVLVLKG-VQCEVKLVESGGG           29

                40        50        60
                |         |         |
10D5vh.pro  ILQSSQTLSLTCSFSGFSLSTSGMGVGWIR          59
3D6vh.PRO   LV-KPGASLKLSCAASGFTFSN--YGMS-WVR        57

                70        80        90
                |         |         |
10D5vh.pro  QPSGKGLEWLAHIYWYDDDKRY-NPSLKSRL         88
3D6vh.PRO   QNSDKRRLEWVASIRSGGGRTYYSDNVKGRF         87

                100       110       120
                |         |         |
10D5vh.pro  TISKDTSRRKQVFLKITSVDPADTATYYCVR         118
3D6vh.PRO   TIHRENAKNTLYLQMSSLKSEDTALYYCVR          117

                130       140
                |         |
10D5vh.pro  RPHI--TPVLSPVDAMDYWGQGTSVTVSS           142
3D6vh.PRO   ---YDHYSGSSDYWGQGTTVTVSS                138
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- 1 -

SEQUENCE LISTING

<110> Neuralab Limited

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<130> ELN-002PC

<150> 60/251,892
<151> 2000-12-06

<160> 63

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<213> Mus musculus

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Met Met Ser Pro Ala Gln Phe Leu Phe Leu Leu Val Leu Trp Ile Arg
-20                 -15                 -10                 -5

gaa acc aac ggt tat gtt gtg atg acc cag act cca ctc act ttg tcg      96
Glu Thr Asn Gly Tyr Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser
                1                   5                   10

gtt acc att gga caa cca gcc tcc atc tct tgc aag tca agt cag agc     144
Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
            15                  20                  25

ctc tta gat agt gat gga aag aca tat ttg aat tgg ttg tta cag agg     192
Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg
        30                  35                  40

cca ggc cag tct cca aag cgc cta atc tat ctg gtg tct aaa ctg gac     240
Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
45                  50                  55                  60

tct gga gtc cct gac agg ttc act ggc agt gga tca ggg aca gat ttt     288
Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
                65                  70                  75

aca ctg aaa atc agc aga ata gag gct gag gat ttg gga ctt tat tat     336
Thr Leu Lys Ile Ser Arg Ile Glu Ala Glu Asp Leu Gly Leu Tyr Tyr
                80                  85                  90

- 2 -

```
tgc tgg caa ggt aca cat ttt cct cgg acg ttc ggt gga ggc acc aag    384
Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys
        95                  100             105

ctg gaa atc aaa                                                     396
Leu Glu Ile Lys
    110
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-20              -15                 -10                     -5
Glu Thr Asn Gly Tyr Val Val Met Thr Gln Thr Pro Leu Thr Leu Ser
                1               5                   10
Val Thr Ile Gly Gln Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
            15                  20                  25
Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Arg
    30                  35                  40
Pro Gly Gln Ser Pro Lys Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
45                  50                  55                  60
Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe
                65                  70                  75
Thr Leu Lys Ile Ser Arg Ile Glu Ala Glu Asp Leu Gly Leu Tyr Tyr
                80                  85                  90
Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gly Gly Thr Lys
            95                  100             105
Leu Glu Ile Lys
    110
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<213> Mus musculus

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atg aac ttc ggg ctc agc ttg att ttc ctt gtc ctt gtt tta aaa ggt    48
Met Asn Phe Gly Leu Ser Leu Ile Phe Leu Val Leu Val Leu Lys Gly
            -15                 -10                 -5

gtc cag tgt gaa gtg aag ctg gtg gag tct ggg gga ggc tta gtg aag    96
Val Gln Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys
            1               5                   10
```

- 3 -

```
cct gga gcg tct ctg aaa ctc tcc tgt gca gcc tct gga ttc act ttc    144
Pro Gly Ala Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        15                  20                  25

agt aac tat ggc atg tct tgg gtt cgc cag aat tca gac aag agg ctg    192
Ser Asn Tyr Gly Met Ser Trp Val Arg Gln Asn Ser Asp Lys Arg Leu
30                  35                  40                  45

gag tgg gtt gca tcc att agg agt ggt ggt ggt aga acc tac tat tca    240
Glu Trp Val Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser
                50                  55                  60

gac aat gta aag ggc cga ttc acc atc tcc aga gag aat gcc aag aac    288
Asp Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn
                65                  70                  75

acc ctg tac ctg caa atg agt agt ctg aag tct gag gac acg gcc ttg    336
Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Leu
            80                  85                  90

tat tat tgt gtc aga tat gat cac tat agt ggt agc tcc gac tac tgg    384
Tyr Tyr Cys Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp
        95                  100                 105

ggc cag ggc acc act gtc aca gtc tcc tca                            414
Gly Gln Gly Thr Thr Val Thr Val Ser Ser
110                 115
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<222> (1)...(19)

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                -15                 -10                 -5
Val Gln Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Lys
            1                   5                   10
Pro Gly Ala Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        15                  20                  25
Ser Asn Tyr Gly Met Ser Trp Val Arg Gln Asn Ser Asp Lys Arg Leu
30                  35                  40                  45
Glu Trp Val Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser
                50                  55                  60
Asp Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Glu Asn Ala Lys Asn
                65                  70                  75
Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Leu
            80                  85                  90
Tyr Tyr Cys Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp
        95                  100                 105
Gly Gln Gly Thr Thr Ile Thr Val Ser Ser
110                 115
```

- 4 -

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<210> 5
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<213> Artificial Sequence

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<222> (1)...(20)

<223> humanized 3D6 light chain variable region

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


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<213> Homo sapiens

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<222> (1)...(19)

<400> 6
Met Gly Leu Leu Met Leu Trp Val Ser Gly Ser Ser Gly Asp Ile Val
            -15                 -10                 -5
Met Thr Gln Ser Pro Leu Ser Leu Pro Val Thr Pro Gly Glu Pro Ala
             1               5                   10
Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Leu His Ser Asn Gly Tyr
    15              20                  25
Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser Pro Gln Leu
30              35                  40                      45
Leu Ile Tyr Leu Gly Ser Asn Arg Ala Ser Gly Val Pro Asp Arg Phe
            50                  55                  60
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Lys Ile Ser Arg Val
            65                  70                  75
Glu Ala Glu Asp Val Gly Val Tyr Tyr Cys Met Gln Ala Leu Gln Thr
        80                  85                  90
Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
    95              100                 105
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<210> 7
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<212> PRT
<213> Homo sapiens

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Asp Ile 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 Leu Leu His Ser
            20                  25                  30
Asn Gly Tyr Asn Tyr Leu Asp Trp Tyr Leu Gln Lys Pro Gly Gln Ser
        35                  40                  45
Pro Gln Leu Leu Ile Tyr Leu Gly Ser Asn Arg Ala 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 Ala
                85                  90                  95
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            100


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<221> SIGNAL
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            -15                 -10                 -5
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
             1                   5                  10
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
    15                  20                  25
Ser Asn Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30                  35                  40                  45
Glu Trp Val Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser
                50                  55                  60
Asp Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn
                65                  70                  75
Ser Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu
            80                  85                  90
Tyr Tyr Cys Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp
    95                  100                 105
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110                 115
```

- 6 -

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<213> Homo sapiens

<400> 9
Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln Pro Gly Gly
 1               5                  10                  15
Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Ser Tyr
            20                  25                  30
Ala Val Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val
            35                  40                  45
Ser Ala Ile Ser Gly Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val
        50                  55                  60
Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Ser Leu Tyr
65                  70                  75                  80
Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Leu Tyr Tyr Cys
                85                  90                  95
Ala Lys Asp Asn Tyr Asp Phe Trp Ser Gly Thr Phe Asp Tyr Trp Gly
            100                 105                 110
Gln Gly Thr Leu Val Thr Val Ser Ser
            115                 120


<210> 10
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<212> PRT
<213> Homo sapiens

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


<210> 11
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<223> humanized 3D6 light chain variable region
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-20               -15               -10                       -5
Glu Thr Asn Gly Asp Val Val Met Thr Gln Ser Pro Leu Ser Leu Pro
                1               5                       10
Val Thr Pro Gly Glu Pro Ala Ser Ile Ser Cys Lys Ser Ser Gln Ser
        15              20              25
Leu Leu Asp Ser Asp Gly Lys Thr Tyr Leu Asn Trp Leu Leu Gln Lys
    30              35              40
Pro Gly Gln Ser Pro Gln Arg Leu Ile Tyr Leu Val Ser Lys Leu Asp
45              50              55                      60
Ser Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe
                65              70              75
Thr Leu Lys Ile Ser Arg Val Glu Ala Glu Asp Val Gly Val Tyr Tyr
            80              85              90
Cys Trp Gln Gly Thr His Phe Pro Arg Thr Phe Gly Gln Gly Thr Lys
        95              100             105
Val Glu Ile Lys
    110


<210> 12
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<221> SIGNAL
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                -15             -10                     -5
Val Gln Cys Glu Val Gln Leu Leu Glu Ser Gly Gly Gly Leu Val Gln
                1               5               10
Pro Gly Gly Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe
        15              20              25
Ser Asn Tyr Gly Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu
30              35                  40                          45
Glu Trp Val Ala Ser Ile Arg Ser Gly Gly Gly Arg Thr Tyr Tyr Ser
                50              55                      60
Asp Asn Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn
                65              70              75
Thr Leu Tyr Leu Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val
        80              85              90
Tyr Tyr Cys Val Arg Tyr Asp His Tyr Ser Gly Ser Ser Asp Tyr Trp
    95              100             105
Gly Gln Gly Thr Leu Val Thr Val Ser Ser
110             115


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<222> (1)...(57)

<400> 13
atg aag ttg cct gtt agg ctg ttg gta ctg atg ttc tgg att cct gct    48
Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
            -15                 -10                 -5

tcc agc agt gat gtt ttg atg acc caa act cca ctc tcc ctg cct gtc    96
Ser Ser Ser Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val
            1                   5                   10

agt ctt gga gat caa gcc tcc atc tct tgc aga tct agt cag aac att   144
Ser Leu Gly Asp Gln Ala Ser Ile Ser Cys Arg Ser Ser Gln Asn Ile
        15                  20                  25

ata cat agt aat gga aac acc tat tta gaa tgg tac ctg cag aaa cca   192
Ile His Ser Asn Gly Asn Thr Tyr Leu Glu Trp Tyr Leu Gln Lys Pro
    30                  35                  40                  45

ggc cag tct cca aag ctc ctg atc tac aaa gtt tcc aac cga ttt tct   240
Gly Gln Ser Pro Lys Leu Leu Ile Tyr Lys Val Ser Asn Arg Phe Ser
                50                  55                  60

ggg gtc cca gac agg ttc agt ggc agt gga tca ggg aca gat ttc aca   288
Gly Val Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr
                65                  70                  75

ctc aag atc aag aaa gtg gag gct gag gat ctg gga att tat tac tgc   336
Leu Lys Ile Lys Lys Val Glu Ala Glu Asp Leu Gly Ile Tyr Tyr Cys
            80                  85                  90

ttt caa ggt tca cat gtt ccg ctc acg ttc ggt gct ggg acc aag ctg   384
Phe Gln Gly Ser His Val Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu
        95                  100                 105

gag ctg gaa                                                        393
Glu Leu Glu
110


<210> 14
<211> 131
<212> PRT
<213> Mus musculus

<220>
<221> SIGNAL
<222> (1)...(19)

<400> 14
Met Lys Leu Pro Val Arg Leu Leu Val Leu Met Phe Trp Ile Pro Ala
            -15                 -10                 -5
Ser Ser Ser Asp Val Leu Met Thr Gln Thr Pro Leu Ser Leu Pro Val
            1                   5                   10
```

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


<210> 15
<211> 426
<212> DNA
<213> Mus musculus

<220>
<221> CDS
<222> (1)...(426)

<221> sig_peptide
<222> (1)...(57)

<400> 15
atg gac agg ctt act tcc tca ttc ctg ctg ctg att gtc cct gca tat      48
Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
              -15                 -10                 -5

gtc ctg tcc cag gct act ctg aaa gag tct ggc cct gga ata ttg cag      96
Val Leu Ser Gln Ala Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln
              1                   5                   10

tcc tcc cag acc ctc agt ctg act tgt tct ttc tct ggg ttt tca ctg     144
Ser Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu
        15                  20                  25

agc act tct ggt atg gga gtg agc tgg att cgt cag cct tca gga aag     192
Ser Thr Ser Gly Met Gly Val Ser Trp Ile Arg Gln Pro Ser Gly Lys
  30                  35                  40                  45

ggt ctg gag tgg ctg gca cac att tac tgg gat gat gac aag cgc tat     240
Gly Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
                50                  55                  60

aac cca tcc ctg aag agc cgg ctc aca atc tcc aag gat acc tcc aga     288
Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg
            65                  70                  75

aag cag gta ttc ctc aag atc acc agt gtg gac cct gca gat act gcc     336
Lys Gln Val Phe Leu Lys Ile Thr Ser Val Asp Pro Ala Asp Thr Ala
            80                  85                  90
```

```
aca tac tac tgt gtt cga agg ccc att act ccg gta cta gtc gat gct   384
Thr Tyr Tyr Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala
     95                  100                 105

atg gac tac tgg ggt caa gga acc tca gtc acc gtc tcc tca           426
Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
110                 115                 120
```

<210> 16
<211> 142
<212> PRT
<213> Mus musculus

<220>
<221> SIGNAL
<222> (1)...(19)

<400> 16
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Met Asp Arg Leu Thr Ser Ser Phe Leu Leu Leu Ile Val Pro Ala Tyr
              -15                 -10                 -5
Val Leu Ser Gln Ala Thr Leu Lys Glu Ser Gly Pro Gly Ile Leu Gln
              1                 5                 10
Ser Ser Gln Thr Leu Ser Leu Thr Cys Ser Phe Ser Gly Phe Ser Leu
     15                  20                  25
Ser Thr Ser Gly Met Gly Val Ser Trp Ile Arg Gln Pro Ser Gly Lys
30                  35                  40                  45
Gly Leu Glu Trp Leu Ala His Ile Tyr Trp Asp Asp Asp Lys Arg Tyr
              50                  55                  60
Asn Pro Ser Leu Lys Ser Arg Leu Thr Ile Ser Lys Asp Thr Ser Arg
              65                  70                  75
Lys Gln Val Phe Leu Lys Ile Thr Ser Val Asp Pro Ala Asp Thr Ala
              80                  85                  90
Thr Tyr Tyr Cys Val Arg Arg Pro Ile Thr Pro Val Leu Val Asp Ala
     95                  100                 105
Met Asp Tyr Trp Gly Gln Gly Thr Ser Val Thr Val Ser Ser
110                 115                 120
```

<210> 17
<211> 136
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 17
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tccgcaagct tgccgccacc atggacatgc gcgtgcccgc ccagctgctg ggcctgctga  60
tgctgtgggt gtccggctcc tccggctacg tggtgatgac ccagtccccc ctgtccctgc 120
ccgtgacccc cggcga                                                 136
```

<210> 18
<211> 131
<212> DNA
<213> Artificial Sequence

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```
<220>
<223> Primer

<400> 18
ctggggggac tggccgggct tctgcagcag ccagttcagg taggtcttgc cgtcggagtc 60
cagcagggac tgggaggact tgcaggagat ggaggcgggc tcgccggggg tcacgggcag 120
ggacaggggg g                                                      131

<210> 19
<211> 146
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 19
acctgaactg gctgctgcag aagcccggcc agtcccccca gcgcctgatc tacctggtgt 60
ccaagctgga ctccggcgtg cccgaccgct tctccggctc cggctccggc accgacttca 120
ccctgaagat ctcccgcgtg gaggcc                                      146

<210> 20
<211> 142
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 20
aattctagga tccactcacg cttgatctcc accttggtgc cctggccgaa ggtgcggggg 60
aagtgggtgc cctgccagca gtagtacacg cccacgtcct cggcctccac gcgggagatc 120
ttcagggtga agtcggtgcc gg                                          142

<210> 21
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 21
ctggggggac tggccg                                                  16

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

<220>
<223> Primer

<400> 22
acctgaactg gctgctgcag aa                                           22

<210> 23
<211> 138
<212> DNA
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```
<213> Artificial Sequence

<220>
<223> Primer

<400> 23
acagaaagct tgccgccacc atggagtttg ggctgagctg gcttttcctt gtggctattt 60
taaaaggtgt ccagtgtgag gtgcagctgc tggagtccgg cggcggcctg gtgcagcccg 120
gcggctccct cgcctgt                                                  138

<210> 24
<211> 135
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 24
gccgccggag cggatggagg ccacccactc caggcccttg ccggggggcct ggcgcaccca 60
ggacatgccg tagttggaga aggtgaagcc ggaggcggcg caggacaggc gcagggagcc 120
gccgggctgc accag                                                    135

<210> 25
<211> 142
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 25
ctggagtggg tggcctccat ccgctccggc ggcggccgca cctactactc cgacaacgtg 60
aagggccgct tcaccatctc ccgcgacaac gccaagaact ccctgtacct gcagatgaac 120
tccctgcgcg ccgaggacac cg                                            142

<210> 26
<211> 144
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 26
ctgcaaggat ccactcaccg gaggacacgg tcaccagggt gccctggccc cagtagtcgg 60
aggagccgga gtagtggtcg tagcgcacgc agtagtacag ggcggtgtcc tcggcgcgca 120
gggagttcat ctgcaggtac aggg                                          144

<210> 27
<211> 16
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 27
gccgccggag cggatg                                                   16
```

```
<210> 28
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 28
ctggagtggg tggcctccat                                              20

<210> 29
<211> 19
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 29
tccgcaagct tgccgccac                                               19

<210> 30
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 30
aattctagga tccactcacg cttgatctc                                    29

<210> 31
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 31
acagaaagct tgccgccacc atg                                          23

<210> 32
<211> 22
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer

<400> 32
ctgcaaggat ccactcaccg ga                                           22

<210> 33
<211> 10
<212> PRT
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<213> Artificial Sequence

<220>
<223> internal peptide

<400> 33
Asp Ala Glu Phe Arg His Asp Ser Gly Tyr
 1               5                   10


<210> 34
<211> 402
<212> DNA
<213> Artificial Sequence

<220>
<223> h3D6 version 1 VL

<400> 34
atggacatgc gcgtgcccgc ccagctgctg ggcctgctga tgctgtgggt gtccggctcc 60
tccggctacg tggtgatgac ccagtccccc ctgtccctgc ccgtgacccc cggcgagccc 120
gcctccatct cctgcaagtc ctcccagtcc ctgctggact ccgacggcaa gacctacctg 180
aactggctgc tgcagaagcc cggccagtcc ccccagcgcc tgatctacct ggtgtccaag 240
ctggactccg gcgtgcccga ccgcttctcc ggctccggct ccggcaccga cttcaccctg 300
aagatctccc gcgtggaggc cgaggacgtg ggcgtgtact actgctggca gggcacccac 360
ttcccccgca ccttcggcca gggcaccaag gtggagatca ag                   402

<210> 35
<211> 402
<212> DNA
<213> Artificial Sequence

<220>
<223> h3D6 version 2 VL

<400> 35
atggacatgc gcgtgcccgc ccagctgctg ggcctgctga tgctgtgggt gtccggctcc 60
tccggcgacg tggtgatgac ccagtccccc ctgtccctgc ccgtgacccc cggcgagccc 120
gcctccatct cctgcaagtc ctcccagtcc ctgctggact ccgacggcaa gacctacctg 180
aactggctgc tgcagaagcc cggccagtcc ccccagcgcc tgatctacct ggtgtccaag 240
ctggactccg gcgtgcccga ccgcttctcc ggctccggct ccggcaccga cttcaccctg 300
aagatctccc gcgtggaggc cgaggacgtg ggcgtgtact actgctggca gggcacccac 360
ttcccccgca ccttcggcca gggcaccaag gtggagatca ag                   402

<210> 36
<211> 414
<212> DNA
<213> Artificial Sequence

<220>
<223> h3D6 version 1 VH

<400> 36
atggagtttg ggctgagctg gcttttttctt gtggctattt taaaaggtgt ccagtgtgag 60
gtgcagctgc tggagtccgg cggcggcctg gtgcagcccg gcggctccct cgcctgtccc 120
tgcgccgcct ccggcttcac cttctccaac tacggcatgt cctgggtgcg ccaggccccc 180
ggcaagggcc tggagtgggt ggcctccatc cgctccggcg gcggccgcac ctactactcc 240
gacaacgtga agggccgctt caccatctcc cgcgacaacg ccaagaactc cctgtacctg 300
cagatgaact ccctgcgcgc cgaggacacc gccctgtact actgcgtgcg ctacgaccac 360

tactccggct cctccgacta ctggggccag ggcaccctgg tgaccgtgtc ctcc          414

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<210> 37
<211> 414
<212> DNA
<213> Artificial Sequence

<220>
<223> h3D6 version 2 VH

<400> 37
atggagtttg ggctgagctg gcttttttctt gtggctattt taaaaggtgt ccagtgtgag 60
gtgcagctgc tggagtccgg cggcggcctg gtgcagcccg gcggctccct gcgcctgtcc 120
tgcgccgcct ccggcttcac cttctccaac tacggcatgt cctgggtgcg ccaggccccc 180
ggcaagggcc tggagtgggt ggcctccatc cgctccggcg gcggccgcac ctactactcc 240
gacaacgtga agggccgctt caccatctcc cgcgacaact ccaagaacac cctgtacctg 300
cagatgaact ccctgcgcgc cgaggacacc gccgtgtact actgcgtgcg ctacgaccac 360
tactccggct cctccgacta ctggggccag ggcaccctgg tgaccgtgtc               

<210> 38
<211> 770
<212> PRT
<213> Homo Sapiens

<400> 38
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Met Leu Pro Gly Leu Ala Leu Leu Leu Leu Ala Ala Trp Thr Ala Arg
 1               5                  10                  15
Ala Leu Glu Val Pro Thr Asp Gly Asn Ala Gly Leu Leu Ala Glu Pro
            20                  25                  30
Gln Ile Ala Met Phe Cys Gly Arg Leu Asn Met His Met Asn Val Gln
        35                  40                  45
Asn Gly Lys Trp Asp Ser Asp Pro Ser Gly Thr Lys Thr Cys Ile Asp
    50                  55                  60
Thr Lys Glu Gly Ile Leu Gln Tyr Cys Gln Glu Val Tyr Pro Glu Leu
65                  70                  75                  80
Gln Ile Thr Asn Val Val Glu Ala Asn Gln Pro Val Thr Ile Gln Asn
                85                  90                  95
Trp Cys Lys Arg Gly Arg Lys Gln Cys Lys Thr His Pro His Phe Val
            100                 105                 110
Ile Pro Tyr Arg Cys Leu Val Gly Glu Phe Val Ser Asp Ala Leu Leu
        115                 120                 125
Val Pro Asp Lys Cys Lys Phe Leu His Gln Glu Arg Met Asp Val Cys
    130                 135                 140
Glu Thr His Leu His Trp His Thr Val Ala Lys Glu Thr Cys Ser Glu
145                 150                 155                 160
Lys Ser Thr Asn Leu His Asp Tyr Gly Met Leu Leu Pro Cys Gly Ile
                165                 170                 175
Asp Lys Phe Arg Gly Val Glu Phe Val Cys Cys Pro Leu Ala Glu Glu
            180                 185                 190
Ser Asp Asn Val Asp Ser Ala Asp Ala Glu Glu Asp Asp Ser Asp Val
            195                 200                 205
Trp Trp Gly Gly Ala Asp Thr Asp Tyr Ala Asp Gly Ser Glu Asp Lys
    210                 215                 220
Val Val Glu Val Ala Glu Glu Glu Val Ala Glu Val Glu Glu Glu
225                 230                 235                 240
Glu Ala Asp Asp Asp Glu Asp Asp Glu Asp Gly Asp Glu Val Glu Glu
                245                 250                 255
Glu Ala Glu Glu Pro Tyr Glu Glu Ala Thr Glu Arg Thr Thr Ser Ile
            260                 265                 270

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Ala Thr Thr Thr Thr Thr Thr Thr Glu Ser Val Glu Glu Val Val Arg
        275                 280             285
Glu Val Cys Ser Glu Gln Ala Glu Thr Gly Pro Cys Arg Ala Met Ile
    290             295                 300
Ser Arg Trp Tyr Phe Asp Val Thr Glu Gly Lys Cys Ala Pro Phe Phe
305             310                 315                 320
Tyr Gly Gly Cys Gly Gly Asn Arg Asn Asn Phe Asp Thr Glu Glu Tyr
            325                 330                 335
Cys Met Ala Val Cys Gly Ser Ala Met Ser Gln Ser Leu Leu Lys Thr
            340                 345                 350
Thr Gln Glu Pro Leu Ala Arg Asp Pro Val Lys Leu Pro Thr Thr Ala
        355                 360                 365
Ala Ser Thr Pro Asp Ala Val Asp Lys Tyr Leu Glu Thr Pro Gly Asp
        370                 375                 380
Glu Asn Glu His Ala His Phe Gln Lys Ala Lys Glu Arg Leu Glu Ala
385                 390                 395                 400
Lys His Arg Glu Arg Met Ser Gln Val Met Arg Glu Trp Glu Glu Ala
            405                 410                 415
Glu Arg Gln Ala Lys Asn Leu Pro Lys Ala Asp Lys Lys Ala Val Ile
            420                 425                 430
Gln His Phe Gln Glu Lys Val Glu Ser Leu Glu Gln Glu Ala Ala Asn
        435                 440                 445
Glu Arg Gln Gln Leu Val Glu Thr His Met Ala Arg Val Glu Ala Met
    450                 455                 460
Leu Asn Asp Arg Arg Arg Leu Ala Leu Glu Asn Tyr Ile Thr Ala Leu
465                 470                 475                 480
Gln Ala Val Pro Pro Arg Pro Arg His Val Phe Asn Met Leu Lys Lys
            485                 490                 495
Tyr Val Arg Ala Glu Gln Lys Asp Arg Gln His Thr Leu Lys His Phe
            500                 505                 510
Glu His Val Arg Met Val Asp Pro Lys Lys Ala Ala Gln Ile Arg Ser
        515                 520                 525
Gln Val Met Thr His Leu Arg Val Ile Tyr Glu Arg Met Asn Gln Ser
    530                 535                 540
Leu Ser Leu Leu Tyr Asn Val Pro Ala Val Ala Glu Glu Ile Gln Asp
545                 550                 555                 560
Glu Val Asp Glu Leu Leu Gln Lys Glu Gln Asn Tyr Ser Asp Asp Val
            565                 570                 575
Leu Ala Asn Met Ile Ser Glu Pro Arg Ile Ser Tyr Gly Asn Asp Ala
            580                 585                 590
Leu Met Pro Ser Leu Thr Glu Thr Lys Thr Thr Val Glu Leu Leu Pro
        595                 600                 605
Val Asn Gly Glu Phe Ser Leu Asp Asp Leu Gln Pro Trp His Ser Phe
    610             615                 620
Gly Ala Asp Ser Val Pro Ala Asn Thr Glu Asn Glu Val Glu Pro Val
625                 630                 635                 640
Asp Ala Arg Pro Ala Ala Asp Arg Gly Leu Thr Thr Arg Pro Gly Ser
            645                 650                 655
Gly Leu Thr Asn Ile Lys Thr Glu Glu Ile Ser Glu Val Lys Met Asp
            660                 665                 670
Ala Glu Phe Arg His Asp Ser Gly Tyr Glu Val His His Gln Lys Leu
            675                 680                 685
Val Phe Phe Ala Glu Asp Val Gly Ser Asn Lys Gly Ala Ile Ile Gly
        690                 695                 700
Leu Met Val Gly Gly Val Val Ile Ala Thr Val Ile Val Ile Thr Leu
705                 710                 715                 720
Val Met Leu Lys Lys Lys Gln Tyr Thr Ser Ile His His Gly Val Val
            725                 730                 735
```

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```
Glu Val Asp Ala Ala Val Thr Pro Glu Glu Arg His Leu Ser Lys Met
            740                 745                 750
Gln Gln Asn Gly Tyr Glu Asn Pro Thr Tyr Lys Phe Phe Glu Gln Met
        755                 760                 765
Gln Asn
    770
```

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<210> 39
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers

<400> 39
acttatatct gtttt                                                    15

<210> 40
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers

<400> 40
acttatacac tttgt                                                    15

<210> 41
<211> 15
<212> DNA
<213> Artificial Sequence

<220>
<223> Primers

<400> 41
acttatgttc attst                                                    15

<210> 42
<211> 16
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 42
acttatgccc attgtt                                                   16

<210> 43
<211> 15
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers
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<400> 43
acttatatat attgt                                                        15

<210> 44
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 44
acttatgkyy attg                                                         14

<210> 45
<211> 15
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 45
acttatgtat acayw                                                        15

<210> 46
<211> 15
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 46
acttatggct ymtct                                                        15

<210> 47
<211> 13
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 47
acttattccc att                                                          13

<210> 48
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 48
acttatatgt tctc                                                         14
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<210> 49
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 49
acttataccc attt                                                          14

<210> 50
<211> 11
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 50
ggacggttgg g                                                             11

<210> 51
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 51
acttataggg tttt                                                          14

<210> 52
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 52
acttatgggr tttt                                                          14

<210> 53
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 53
acttatatgt agtt                                                          14

<210> 54
<211> 13
<212> DNA
<213> <213> Artificial Sequence
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<220>
<223> Primers

<400> 54
acttatatgt gtt                                              13

<210> 55
<211> 15
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 55
acttatacgt atttt                                           15

<210> 56
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 56
acttatctts tttg                                            14

<210> 57
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 57
acttatrggg tttt                                            14

<210> 58
<211> 13
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 58
acttatgttt ttg                                             13

<210> 59
<211> 15
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers
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<400> 59
acttatmgtg mtttt                                                      15

<210> 60
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 60
acttatggtc tctt                                                      14

<210> 61
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 61
acttatatgt tttt                                                      14

<210> 62
<211> 14
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 62
acttattttg ttat                                                      14

<210> 63
<211> 11
<212> DNA
<213> <213> Artificial Sequence

<220>
<223> Primers

<400> 63
ggacgggaca g
```