Title: METHODS OF TREATMENT OR PROPHYLAXIS OF AMYLOIDOGENIC DISEASES OF THE EYE OR OPTIC NERVE

Abstract: The invention provides compositions and methods for the prophylactic or therapeutic treatment of amyloidogetic diseases of the eye including the optic nerve. Such methods comprise administering a therapeutic agent that reduces or eliminates optic amyloid deposits. The methods are especially useful for the therapeutic intervention and prophylaxis of Age-Related Maculopathy (ARM), Age-Related Macular Degeneration (ARMD), or Glaucomatous Optic Neuropathy (GON).
TITLE OF THE INVENTION
METHODS OF TREATMENT OR PROPHYLAXIS OF AMYLOIDOGENIC DISEASES OF THE EYE OR OPTIC NERVE

5 CROSS REFERENCE TO RELATED APPLICATIONS
This application claims the benefit of U.S. Provisional Application No. 60/615,291 filed October 1, 2004, the contents of which are incorporated herein by reference in their entirety.

10 FIELD OF THE INVENTION
This invention relates to therapies for the treatment or prophylaxis of amyloidogenic diseases of the eye or optic nerve.

BACKGROUND OF THE INVENTION
The macula is located within the posterior pole of the eye, a region of the retina most easily viewed with a direct ophthalmoscope. The macula is circular and has a diameter of about 6 mm. The fovea lies in the center of the macula with vascular arcades of the central retinal artery and vein arching around it. The highest concentration of cones and the highest densities of ganglion cells are found within the macula. Visual discrimination with high resolution of fine detail and color depend on the high densities of cones and ganglion cells in the macula. Many diseases can impact the macula.

Several diagnostic terms are used to describe age-related macular changes. The early course of age-related macular disease is called age-related maculopathy (ARM) or sometimes “early age-related macular degeneration” (early-ARMD). Late stage age-related macular degeneration is referred to as ARMD, “late ARM”, or simply AMD, Smith, W., et al., Arch. Ophthalmol. 116:583-587 (1998).

The latter three are synonymous and refer to geographic atrophy also known as non-exudative ARMD, neovascular age-related changes within the macula, or exudative ARMD.

ARM and ARMD affect primarily the retinal cones, which in turn leads to deterioration of central vision and with it the ability to read the fine print, recognize faces, and drive a car. Despite the serious erosion of central vision, even patients with advanced ARMD in both eyes often maintain sufficient peripheral vision to walk unaided although their quality of life is seriously reduced.

ARM is the most common cause of irreversible vision loss among the elderly. It is expected that with an aging population the incidence of ARMD will increase. Current treatments are inadequate and would be improved by the development of therapies that address the underlying pathology as well as the progression of the disease. When ARM progresses to become ARMD, the choriocapillaris, Bruch’s membrane, retinal pigmented epithelial (RPE) cell layer and the photoreceptors (rods and cones) are adversely affected.

Histopathologic studies have documented significant and widespread abnormalities in the extracellular matrices associated with the retinal pigment epithelium (RPE), choroid and photoreceptors
of aged individuals and of those with clinically diagnosed ARMD, Sarks, S. H., Br. J. Ophthalmol. 60:324-341 (1976); Sarks, S. H., et al., Eye 2:552-577 (1988); Green, W.R. and Enger, C., Ophthalmology 100:1519-1535 (1993). The most prominent abnormality in the extracellular matrix is drusen. Drusen appear as whitish-yellowish spots that are distal to RPE cells, i.e. they are found in the potential space between the RPE and Bruch’s Membrane (BM), a space also known as the sub-RPE space. Only drusen that are larger than about 25 micrometers in diameter are visible ophthalmoscopically. The following are the common types of drusen:

- Soft distinct drusen (uniform density with sharp edges);
- Soft indistinct drusen (density highest in center with decreasing density outwards towards their fuzzy edges);
- Hard drusen (present in subjects both with and without macular disease); and
- Drusen confluence (any touching or merging of two or more drusen).

Drusen consist of extracellular deposits that accumulate external to the RPE and on the inner collagenous layer of Bruch’s Membrane (BM), Mullins, R. F., et al., FASEB Journal 14: 835-846 (May 2000).

Drusen may be classified morphologically either as hard or soft. Hard drusen tend to be smaller and have distinct margins compared with soft drusen, which tend to be larger and have sloping/indistinct borders. Hard drusen are typically yellow, round, well-demarcated lesions, less than 64 μm in diameter. Zarbin, M., Eur. J. Ophthalmol. 8:199-206 (1998). In small numbers, hard drusen are not considered as a risk factor for the development of ARM/ARMD, Sarks, S. H., et al., Br. J. Ophthalmol. 83:358-368 (1999). However, excessive numbers of hard drusen at a relatively young age may predispose towards RPE atrophy. Conversely, soft drusen have poorly demarcated boundaries and appear pale yellow. By definition they are larger (i.e. greater than 63 μm). Soft drusen are associated with diffuse thickening of the inner aspect of Bruch’s membrane and, with increasing age, tend to become calcified or filled with cholesterol, rendering them crystalline or polychromatic in appearance, Ambati, J., et al., Surv. Ophthal. 48: 257-293 (May-June 2003). Various studies have identified the presence of soft drusen as a strong risk factor for the development of ARM, Bressler, N.M., et al., Retina 14:130-142 (1994); Bressler, N.M., et al., Arch. Ophthalmol. 113:301-308 (1995).

The composition of drusen has recently been explored and it has been found that for some ARMD retinas, but not in the drusen found in normal retina, amyloid beta, the major extracellular deposit in Alzheimer’s disease plaques, is present, Johnson, L.V. et al., P.N.A.S. 99: 11830-11835 (Sept. 3, 2002). Of those with amyloid beta-containing drusen, the largest quantity of amyloid beta-positive vesicles were found at the edges of atrophy. These edges were the regions at greatest risk for expansion of geographic atrophy. Amyloid beta in drusen correlated with the locus of degenerating RPE cells and photoreceptors. Amyloid beta vesicles were absent in areas where the RPE and photoreceptors already died. Combined, these findings suggest that amyloid beta-containing vesicles may be cleared. Thus, amyloid beta is one candidate activator of the complement cascade in the context of drusen formation and

The present invention is directed to compositions and methods for treating or preventing the progression of ophthalmic diseases characterized by amyloid deposition including ARM, ARMD, and also glaucoma.

SUMMARY OF THE INVENTION

The invention provides for compositions and methods to treat or to prevent the progression of an ophthalmic disease characterized by amyloid deposition in the eye or optic nerve in a patient or mammal.

One embodiment of the invention comprises a therapeutic agent that can be used for the passive immunization of the patient or mammal to treat an ophthalmic disease characterized by amyloid deposition in the eye or optic nerve in a patient or mammal. The therapeutic agent of this embodiment comprises a monoclonal antibody, a polyclonal antibody, or a fragment thereof capable of binding with amyloid-beta components within the retina or optic nerve of patients or mammals.

Another embodiment of the invention is the passive immunization of a patient or a mammal to prevent the progression of an ophthalmic disease characterized by amyloid deposition in the eye or optic nerve in a patient or mammal. Still another embodiment of the invention is a method for treating or preventing the progression of an amyloidogenic disease of the eye or optic nerve, including, but not limited to, age-related macular degeneration, age-related maculopathy, glaucomatous optic neuropathy (GON) or retinal or optic degeneration characterized by drusen deposition.

DETAILED DESCRIPTION OF THE INVENTION

The invention provides pharmaceutical compositions for therapeutic intervention and prophylaxis of diseases affecting the eye or optic nerve characterized by the accumulation of amyloid deposits and methods of employing said compositions. Amyloid deposits are insoluble deposits and the specific amyloid peptide component differs in different diseases. It can form droplets, sheets, or possibly both. Amyloid has been identified in the drusen associated with ARM and ARMD and has been found in the optic nerves of patients with glaucomatous optic neuropathy (GON).

Definitions

The term "antibody" as used herein refers to intact antibodies and binding fragments of intact antibodies. Typically, fragments compete with intact antibody from which they were derived for specific binding to an antigen fragment including separate heavy chains, light chains Fab, Fab', F(ab')2, Fabc, and Fv. The term antibody also includes one or more immunoglobulin chains that are chemically conjugated to, or expressed as, fusion proteins with other proteins. The term antibody may also refer to a bispecific antibody. The terms "immunoglobulin" and "antibody" are used interchangeably and refer to
an antigen-binding protein with four-polypeptide chain structure consisting of two heavy and two light chains. The chains can be stabilized by interchain disulfide bonds. The term “domain” refers to a globular region of a heavy or light chain polypeptide comprising peptide loops. Domains are further classified as “constant” or “variable”. Constant domains have a relative lack of sequence variation within the domains of various class members. Variable domains have significant variation within the domains of various class members. Constant domains on the light chain are referred to as CL domains. Constant domains on the heavy chain are referred to as CH domains. Variable domains on the light chain are referred to as VL domains. Variable domains on the heavy chain are referred to as VH domains.

The terms “bispecific” or “bifunctional” with respect to an antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. An intact antibody has two binding sites. In most antibodies, these binding sites are the same. In the case of bispecific and bifunctional antibodies, the two binding sites are different.

The terms “humanized immunoglobulin” or “humanized antibody” refers to an immunoglobulin or antibody that includes at least one humanized immunoglobulin or antibody chain. The term, “humanized immunoglobulin chain” or “humanized antibody chain” refers to an immunoglobulin or antibody chain with a variable region that includes a variable framework region substantially from a human immunoglobulin or antibody and at least one complementarity determining region (CDR), the site that recognizes and binds an antigen, substantially from a non-human immunoglobulin or antibody.

The terms “chimeric immunoglobulin” or “chimeric antibody” refers to an immunoglobulin or antibody whose light and heavy chains are derived from different species. Chimeric immunoglobulins or antibodies can be constructed with genetic engineering methods known to those of ordinary skill in the art using immunoglobulin gene segments belonging to different species.

The term “epitope” refers to a site on an antigen to which the immunoglobulin or antibody binds. Epitopes are also known as antigenic determinants. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary protein folding.

The term “specific binding” between two molecular entities refers to an affinity of at least $10^6$, $10^7$, $10^8$, $10^9$ M$^{-1}$, or $10^{10}$M$^{-1}$. Preferably, the affinities are greater than $10^8$M$^{-1}$.

The term “immune response” refers to the development of a humoral response, that is induced by an antibody, and/or a cellular response, that is induced by antigen specific T cells, and directed against an amyloid in a patient. As intended herein, the response can be an active response induced by the administration of an antigen, i.e. the therapeutic agent, or a passive response, elicited by the administration of a therapeutic agent in the form of an antibody or an immunogenic fragment thereof.

The term “therapeutic agent” refers to any composition of the invention that is used for therapeutic or prophylactic treatment of the ophthalmic diseases herein. Such agents include, but are not limited to, antibodies, fragments of antibodies, or any composition of the invention capable of treating the
symptoms of a patient having an ophthalmic disease characterized by amyloid deposition or preventing the development or progression of such a disease.

The term “amyloid-beta” refers to one protein component found in drusen that has been identified in patients who had non-exudative and also exudative Age Related Macular Degeneration (ARMD), Dentichev, T., et al., Mol. Vision, 9:184-190 (2003).

The term “patient” as used herein refers to a human patient as well as other mammals in need of either prophylactic or therapeutic treatment for the diseases herein.

Amyloid-beta

Amyloid-beta is a small hydrophobic peptide with N and C-terminal heterogeneity. Amyloid-beta is proteolytically released from the amyloid-beta precursor protein (APP), a membrane glycoprotein of unknown function, Selkoe, D. and Schenk, D., Annu. Rev. Pharmacol. Toxicol., 43:545-584 (2003). The peptide has two principal lengths, Amyloid-beta_{42} and amyloid-beta_{40}. Amyloid-beta_{42} is believed to be actively neurotoxic and more commonly present than amyloid-beta_{40} in the brains of patients with Alzheimer’s disease (AD).

In AD, neuritic plaques in the brain are roughly spherical deposits of amyloid-beta protein that are surrounded by activated microglia and reactive astrocytes. Amyloid-beta also accumulates in the small blood vessels of meningeal small vessels, mostly in the outer walls of arterioles and capillaries. In brains of AD patients, deposits of amyloid-beta have been classed morphologically into diffuse, fibrillar, dense-cored (classic), and, compact (burnt-out), Reilly, J., et al., P.N.A.S. 100:4837-4842, 2003. The authors note that these different classes of amyloid-beta can be of potential clinical significance because each may be associated with a varying level of toxicity.

Drusen

In a recent study of adult human eyes processed within four hours of death, 82/152 (54%) had clinical evidence of ARMD in the form of geographic atrophy, choroidal neovascularization, macular drusen, or all three, Anderson D., et al., Exp. Eye Res. 78:243-256, 2004. Spherical domains of amyloid-beta ranging from 0.25 to 10 micrometers in diameter were commonly present within drusen and as discrete elements within the sub-RPE space, Anderson D., et al., Exp. Eye Res. 78:243-256, 2004. A single druse could contain as few as one or greater than a hundred of these spheres Anderson D., et al., Exp. Eye Res. 78:243-256, 2004. The larger spheres consist of a lucent core surrounded by multiple, concentric inner rings and a dense outer shell. Smaller spheres consist of a core, a single inner ring, and an outer shell, and fusion of the spheres can occur, Anderson D., et al., Exp. Eye Res. 78:243-256, 2004. A separate study with a small sample size showed that amyloid-beta positive drusen were not present in retinas harvested from patients who did not have ARMD. Patients with early ARMD had a few amyloid-beta positive drusen. Patients with geographic atrophy (GA), an advanced form of non-exudative ARMD, had many amyloid-beta positive drusen and each contained many amyloid-beta containing vesicles, Dentichev T., et al., Mol. Vision 9:184, 2003. The occurrence of amyloid-beta drusen was especially...

These spheres of amyloid-beta are not rich in either cholesterol or lipid. The outer most layer of the spheres is associated with amyloid-beta whereas activation-specific complement fragments (iC3b, C3d) are associated with the concentric inner rings. Ultrastructural analysis revealed the spheres to be complex assemblies rather than membrane-bound structures, Anderson, D., et al., Exp. Eye Res. 78:243-256, 2004. Drusen are also associated with inflammatory proteins including terminal complement components, the membrane attack complex (MAC i.e. C5b, C6, C7, C8, C9), inhibitors of complement activation, and several possible complement-activating molecules, Johnson, L., et al., 2002, supra. The significance of the iC3b-rich inner sphere is currently unknown.

Amyloid-beta assemblies appear to originate from degenerating RPE cells that become sequestered in the sub-RPE space; a process that precedes the formation of mature drusen and constitute a chronic inflammatory stimulus and potential nucleation site for drusen to form. Anderson, D., et al., Am J Ophthalmol. 134:411-431, 2002. RPE cells are derived from neuro-ectoderm and therefore are considered to be cells of the central nervous system. The amyloid-beta does not appear to be derived from the blood but rather the overlying RPE cells, i.e. amyloid-beta and amyloid precursor protein both have a local source Detchev T., et al., supra.

The spherical assemblies identified in the sub-RPE space do not bear a resemblance to the amyloid-beta plaques identified in AD brain, thus the amyloid assemblies in drusen are not the ocular equivalent of senile plaques in AD brain. Rather the amyloid-beta assemblies in drusen are similar to those that assemble spontaneously in a cell-free system. Thus, one object of the present invention is to attack, hydrolyze, and disperse any amyloid-beta assemblies found within the sub-RPE space, retina, optic nerve, or neurons along the optic radiations emanating out from the lateral geniculate nucleus.

Amyloid-beta assemblies are a common component of drusen. In donors with a low drusen load (one to five drusen/mm), about 50% of donors had amyloid-beta assemblies. In donors with a moderate to heavy drusen load (greater than twenty drusen/mm), about 100% of donors had amyloid-beta assemblies, Anderson D., et al., 2004, supra. Similar to treatment strategies for Alzheimer’s Disease, treatment strategies in ARM and ARMD include not only diminishing or clearing the amyloid-beta deposits that have already accumulated, but also decreasing the production of new amyloid-beta in the sub-RPE space.

Rationale for treating diseases of the optic nerve including glaucomatous optic neuropathy


Glaucoma and primate models of glaucoma have been shown to be associated with deficiency of anterograde transport from the RGC cell body in the retina to its synapse in the lateral geniculate nucleus in the brain, which may relate to an increased susceptibility to excitotoxic damage, Naskar, R., et al., Invest. Ophthalmol. Vis. Sci., 41:1940-1944 (2000), Minckler, D.S., et al., Invest. Ophthalmol. Vis. Sci., 16:426-441(1977) and Anderson, D. R., and Hendrickson, A., Invest. Ophthalmol. Vis. Sci., 13:771-783 (1974). Because this transport mechanism appears to be obstructed in glaucoma, it is possible that glaucoma may be associated with an increase in retinal APP levels at the level of the RGC cell body, although this has not been demonstrated to date.

participate in a positive feedback loop, with further caspase activation, APP cleavage, and the formation of cytotoxic amyloid-beta. Excess amyloid-beta has been found in the aqueous humor of glaucoma patients as well as in those with pseudoexfoliation glaucoma, Janciauskiene, S., and Krakau, T., Doc. Ophthalmol., 106:215-223 (2003), although it has not yet been functionally associated with glaucoma.

A mechanism that allows the accumulation of APP is associated with glaucoma, and such accumulation has been shown in in vitro models to increase the levels of amyloid-beta in neurons. Excess levels of amyloid-beta have been found in glaucomatous eyes, and excess levels of amyloid-beta are toxic to central nervous system neurons including RGCs, Anderson, supra. Without wishing to be bound by any theory, it is believed that amyloid-beta plays a substantial role in the pathology of glaucoma.

Monoclonal antibodies that bind to amyloid-beta could reduce amyloid-beta that has already formed at the level of the RGC and decrease its production within the RGCs, thereby interrupting the process that leads to RGC death in glaucomatous optic neuropathy.

Diagnostic kits

The invention further provides for diagnostic kits to perform the diagnostic methods described above. To detect antibodies, labeled anti-idiotypic antibodies are used. The agent can be supplied pre-bound to a solid phase, such as wells of a microtiter dish.

Kits also typically contain labeling to provide directions on how to use the kit. The labeling may also include a chart or other visual aid that correlates the levels of measured label with levels of antibodies. The term “label” may include any written or recorded material that is attached to, or otherwise accompanies a kit at any time during its manufacture, transport, sale or use. The term “label” also includes, leaflets, brochures, educational materials, promotional materials, packaging materials, and the writing imprinted directly on the kits. The term “label” also includes audio media, video media, computer discs of any sort, and web-based materials.

Therapeutic agents

One embodiment of the invention comprises a therapeutic agent that can be used for the passive immunization of the patient or mammal to treat an ophthalmic disease characterized by amyloid deposition in the eye, optic nerve, or along the anterior or posterior visual pathways in a patient or mammal. Induction of an immune response using the invention is passive since the administered monoclonal or polyclonal antibodies, or an immunogenic fragment thereof, will bind to amyloid-beta within the patient. The therapeutic agent of this embodiment comprises a natural or synthetic immunogen of amyloid beta or a related immunoconjugate, which may be a monoclonal antibody, polyclonal antibody, a fragment thereof, or a nucleic acid molecule that is capable of binding specifically with amyloid-beta components within the retina, optic nerve, or visual pathways of patients or mammals.

Another embodiment of the invention comprises a therapeutic agent that can be used for the active immunization of the patient or mammal to treat an ophthalmic disease characterized by amyloid deposition in the eye, optic nerve, or along the anterior or posterior visual pathways in a patient or mammal.
mammal. Such immunization comprises the administering fragments of amyloid beta or derivatives thereof to elicit an immune response against at least one epitope with amyloid beta. The therapeutic agent of this embodiment comprises a natural or synthetic fragment of amyloid beta. Preferably said fragment comprises an N-terminal segment of at least residues 1-12 of amyloid beta, the first residue of amyloid beta being the N-terminal residue of said fragment. Such immunization method comprises the administration to a patient or mammal an effective dose of an agent that induces an immune response against the administered fragment of amyloid beta.

Therapeutic agents contemplated by the invention typically are those of a substantially pure form. This means that an agent is typically at least 50% pure on weight/weight (w/w) basis, as well as being substantially free from interfering proteins and contaminants. In a preferred form, the therapeutic agent is at least 80% w/w pure and, more preferably, at least 90% w/w pure.

A. General description of antibodies

The basic antibody structural unit is the tetramer. Each tetramer is composed of two identical pairs of polypeptide chains. Each pair has one light (ca. 25 kDa) and one heavy chain (ca. 50 to 70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 or more amino acids that are especially 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, mu, alpha, delta, or epsilon; these are about 450 to 600 residues in length and define the antibody’s isotype as IgG, IgM, IgA, IgD, or IgE respectively.

B. Amyloid-beta antibodies

Therapeutic agents of the invention include antibodies that specifically bind to amyloid-beta or other components of amyloid. These antibodies can be monoclonal or polyclonal. Some antibodies bind specifically to the aggregated form of amyloid-beta 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. Human isotype IgG1 is preferred because it has the highest affinity of human isotypes for the FcRlI receptor on phagocytic cells. Bispecific Fab fragments can also be used; one arm of the antibody has specificity for amyloid-beta and the other for an Fc receptor.

The present invention features methods to prevent and treat diseases of the eye, retina, optic nerve, and visual pathways. The invention is based, in part, on the characterization of two monoclonal immunoglobulins, 3D6 and 10D5. Without wishing to be bound by any theory, it is believed that these antibodies effectively bind amyloid-beta and reduce the load of amyloid-beta in the portions of the body related to vision as described above.

Therapeutic immunoglobulins featured in the present invention include a humanized variable light chain, humanized variable heavy chain, or both. Preferred variable light chain, variable heavy chains, or both include a complementarity determining region (CDR) from the monoclonal immunoglobulin and variable frame-work regions substantially from a human acceptor immunoglobulin. “Substantially from a human acceptor immunoglobulin” as used in this context 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; that is, substitutions that alter the 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 a variable region CDR 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 back mutated to a corresponding murine residue, wherein the back mutation does not substantially affect the ability of the chain to direct amyloid-beta binding.

In said embodiment, the invention features a humanized immunoglobulin light or heavy chain that includes 3D6 variable region CDRs 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, 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 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 10D5 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, 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 variable region CDRs and variable framework regions from a human acceptor immunoglobulin light or heavy chain 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.

The present invention also provides for a method to identify 3D6 or 10D5 residues amenable to substitution when producing a humanized 3D6 or 10D5 immunoglobulin, respectively. This includes a method for identifying variable framework region residues that are amenable to substitution using three dimensional structures of the 3D6 or 10D5 variable region on a solved homologous immunoglobulin structure.

C. Production of non-human antibodies

Production of non-human antibodies, e.g. murine, guinea pig, primate, rabbit or rat, can be accomplished by immunizing the animal with amyloid-beta. Mice are typically used for making monoclonal antibodies. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Antibodies are screened for specific binding to amyloid-beta. The smallest fragment to show specific
binding to the antibody defines the epitope of the antibody. Mouse isotype IgG2a is the equivalent of human isotype IgG1.

D. Production of chimeric and humanized antibodies

Chimeric and humanized antibodies have the same or similar binding affinity and specificity as a mouse or other nonhuman antibody. Chimeric antibodies are antibodies whose light and heavy chain genes have been constructed, typically by genetic engineering, from immunoglobulin gene segments belonging to different species. As an example, the variable (V) segments of the genes from a mouse monoclonal antibody may be joined to human constant (C) segments. Human isotype IgG1 is preferred. The typical chimeric antibody is thus a hybrid protein consisting of the V or antigen-binding domain from a mouse antibody and the C segment from a human antibody.

Humanized antibodies have variable region framework residues substantially from a human (acceptor antibody) and complementarity determining regions (CDRs) substantially from a mouse antibody. The constant region(s) if present are substantially or entirely from a human immunoglobulin. The human variable domains are usually chosen from human antibodies whose framework sequences exhibit a high degree of sequence identity with the murine variable region domains from which the CDRs were derived.

Humanized forms of mouse antibodies can be generated by linking the CDRs of non-human antibodies to human constant regions by recombinant DNA methods. Human monoclonal antibodies can be made using phage-display methods. Human antibodies against Amyloid-beta can also be produced from non-human transgenic mammals that have transgenes that encode at least a segment of the human immunoglobulin locus and an inactivated endogenous immunoglobulin locus. Human antibodies can be selected by competitive binding and other studies to have the same epitope specificity as a particular antibody.

Human polyclonal antibodies of the invention can be derived from humans immunized to an immunogenic agent. These can be provided in the form of humans immunized with an immunogenic agent. Polyclonal antibodies can be concentrated by affinity purification using amyloid-beta as an affinity reagent.

Some anti-amyloid-beta antibodies that can be used for the invention described herein bind to epitopes within residues 1-5 (3D6), 3-7 (12B4) or 5-11 (6E10) of amyloid-beta or an epitope comprising a free N-terminal of amyloid-beta. Such an antibody does not necessarily bind to every residue nor does every single amino acid substitution or deletion significantly affect binding affinity. The monoclonal antibodies that bind to a specific epitope within amyloid beta can be a conformational or non-conformational epitope.

Human or humanized antibodies of the invention can be designed with an IgG, IgD, IgA, and IgE constant region and any isotype including IgG1, IgG2, IgG3, and IgG4. Antibodies are usually tetramers with two light and two heavy chains. Human isotype IgG1 is preferred because it has the highest affinity of human isotypes for the FcR1 receptor on phagocytic cells. Humanized forms of mouse
antibodies can be generated by linking CDRs of non-human antibodies to human constant regions using recombinant DNA techniques, Queen et al., P.N.A.S., 86:10029-100033 (1989).

All antibody preparations for the instant invention are purified to ensure low levels of endotoxin. Monoclonals can be prepared against a fragment by injecting a fragment or longer form of amyloid-beta into a mouse and then prepare hybridomas and screen the hybridomas for an antibody that specifically bind to a desired fragment of amyloid-beta without binding to other non-overlapping fragments of amyloid beta.

Human antibodies against amyloid-beta can be made using several methods; these include the following:

1) Trioma methodology

The basic approach using the cell fusion partner SPAZ-4 has been described, Oestberg et al., Hybridoma, 2:361-367 (1983). The antibody-producing cell lines obtained by this method are called triomas because they have 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 hybrid cell, like the SPAZ-4 cell line. This cell is then fused with an immunized human B-lymphocyte to obtain an antibody—producing trioma cell line. The immunized B-lymphocytes are obtained from blood, spleen, lymph nodes or bone marrow of a human donor. Immunization can be either in vivo or in vitro. For in vivo immunization, B cells are usually isolated from a human immunized with amyloid-beta, a fragment of amyloid-beta, a larger polypeptide containing amyloid-beta or a fragment, or an anti-idiotypic antibody to an antibody to amyloid-beta. In some methods, B cells are isolated from the same patient who will receive the antibody therapy. For in vitro immunization, B-lymphocytes are usually exposed to antigen for about 7 to 14 days in a media like RPMI-1640, Engleman, E.G., U.S. Pat. No. 4,634,666, supplemented with human plasma.

The immunized B-lymphocytes are fused to a xenogeneic hybrid cell such as SPAZ-4 using known methods. Clones secreting antibodies that have the required binding specificity are identified by assaying the trioma culture medium for the ability to bind to amyloid-beta or an amyloid-beta fragment. Triomas that produce human antibodies with the desired specificity are sub-cloned by the limiting dilution technique and grown in vitro. Trioma cell lines obtained are then tested for their ability to bind amyloid-beta or a fragment of amyloid beta.

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

2) Transgenic non-human mammals

Human antibodies against amyloid beta can also be produced from non-human transgenic mammals having transgenes that encode at least a segment of the human immunoglobulin locus. Usually, the endogenous immunoglobulin locus of the transgenic mammals is functionally inactivated. Preferably
the segment of the human immunoglobulin locus includes un-rearranged sequences of heavy and light chain components.

Both inactivation and endogenous immunoglobulin genes and introduction of exogenous immunoglobulin genes can be achieved by targeted homologous 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. Transgenic mice are particularly suitable.

Monoclonal antibodies are prepared by 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. Polyclonal antibodies can be concentrated by affinity purification using amyloid-beta or other amyloid peptide as an affinity reagent.

3) Phage display methods

A further approach for obtaining human anti-amyloid-beta antibodies is to screen a DNA library from human B cells according to the general protocol outlined by Huse et al., Science 246:1285-1281 (1989). As described in the trioma methodology (above), such B cells can be obtained from a human immunized with amyloid-beta fragments, longer polypeptides containing amyloid-beta or fragments, or anti-idiotypic antibodies. In some methods, B cells are isolated from the same patient who will receive the antibody therapy. Antibodies binding to amyloid-beta or a fragment of amyloid-beta are then cloned and amplified. The protocol described by Huse is rendered more efficient in combination with phage-display technology. Phage displaying antibodies with a desired specificity are selected by affinity enrichment to an amyloid-beta peptide or fragment of amyloid-beta.

In a variation of the phage-display method, human antibodies having the binding specificity of a selected murine antibody can be produced. Using this method, either the heavy or light chain variable region of the selected murine antibody is used as 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 and a different heavy chain variable region. The heavy chain variable regions are obtained from a library of rearranged human heavy chain variable regions. A phage showing strong specific binding for amyloid-beta (at least $10^9$ and preferably at least $10^9$ M$^{-1}$) is selected.

The human heavy chain variable region from this phage then serves as starting material for constructing a further phage library. In this library, each phage displays the same heavy chain variable region (the region identified from the first 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. Phage showing strong specific binding for amyloid-beta are selected; these phage display the variable regions of completely human anti-amyloid beta antibodies. These antibodies usually have the same or similar epitope specificity as the murine starting material.
E. Selection of the Constant Region

The heavy and light chain variable regions of chimeric, humanized, or human antibodies can be linked to at least a portion of a human constant region (Fc). 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. Usually, the antibody will contain both light chain and heavy chain constant regions. The heavy chain constant region usually includes C_{H1}, hinge, C_{H2}, and C_{H3} 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 on whether antibody-dependent complement, cellular-mediated toxicity, or both are desired. For example, isotypes IgG1 and IgG3 have complement activity and isotypes IgG2 and IgG4 do not. Choice of human isotype IgG1 is preferred since it impacts passage of the antibody into the brain. The humanized antibody may comprise sequences from more than one class or isotype. Light chain constant regions can be lambda or kappa. 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.

F. Cloning and sequencing of the variable regions of mouse 3D6

The nucleotide sequence for a leader peptide of the mouse 3D6 variable heavy chain is as follows:

ATGAACCTCGGCTGAGC TTGATTTTCT
TGCTCTTGTTAAAAAGGTGTCAGTG (SEQ. ID NO.: 1)

The nucleotide sequence for a leader peptide of the mouse 3D6 variable light chain is as follows:

ATGATGAGTCCTGCCCAGTTCTGTTTCTG
TATGCTCTGGGATTCGGAAAACCAACGCT (SEQ. ID NO.: 2)

G. Cloning and sequencing of the variable regions of mouse 10D5

The nucleotide sequence for a leader peptide of the mouse 10D5 variable heavy chain is as follows:

ATGGACAGGGCTTACTTCTCATCCTGC
TGCTGATTGTCCTGCATATGTCCGTCC (SEQ. ID NO.: 3)

The nucleotide sequence for a leader peptide of the mouse 10D5 variable light chain is as follows:

ATGAAGTTGCCTGTTAGGGCTGTTGAC
TGATGTTCTGAGATTCCCTGCTTCCAGCT (SEQ. ID NO.: 4)
H. Expression of recombinant antibodies

Chimeric, humanized, and human antibodies are typically produced by recombinant expression. Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally-associated or heterologous promoter regions. 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 of the cross-reacting antibodies. E. Coli is one prokaryotic host particularly useful for cloning the DNA sequences of the present invention. Microbes such as yeast are also useful for expression. Saccharomyces is a preferred yeast host with suitable vectors having expression control sequences, an origin of replication, and termination sequences as desired.

Mammalian cells are a preferred host for expressing nucleotide segments encoding immunoglobulins or fragments of immunoglobulins. A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include CHO cell lines, Cos cell lines, HeLa cells, transformed B cells, and myeloma cell lines. Preferably the cells are nonhuman.

Alternatively, antibody coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in milk of the transgenic animal. Suitable transgenes include coding sequences for light, heavy, or both chains in operable linkage with a promoter and enhancer from a mammary gland specific gene.

The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host. Once expressed, antibodies can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis, and other methods of protein purification.

I. Production of non-human antibodies

Non-human monoclonal antibodies like mouse, guinea pig, primate, rabbit, or rat can be produced by immunizing the animal with amyloid-beta, a longer polypeptide comprising amyloid-beta, an immunogenic fragment of amyloid-beta, or anti-idiotypic antibodies to an antibody to amyloid-beta.

Mice are typically used to make monoclonal antibodies. Rabbits or guinea pigs are typically used to make polyclonal antibodies. Monoclonals can be prepared against a fragment by injecting the fragment or longer form of amyloid-beta into a mouse, preparing hybridomas and screening the hybridomas for antibodies that specifically bind to amyloid-beta.

J. Production of chimeric antibodies

Chimeric antibodies are hybrid antibodies generated by splicing the gene sequence coding for a non-human derived variable region (containing the CDRs that bind amyloid-beta) to a nucleotide sequence that codes for a constant regions of a human antibody, Walsh, G., Eur. J. Pharm. Biopharm. 58:185-96 (2004). A chimeric antibody can activate the human immune system effector
functions (associated with the Fc region) and displays a circulatory half-life of about 250 hours compared with 40 hours in the case of intact murine antibodies.

K. Production of humanized antibodies

Generally, producing humanized antibodies is a favored approach for antibody engineering. Humanization involves production of a non-human monoclonal antibody against the target antigen, isolation of the nucleotide sequence coding for the CDR, and the use of the isolated sequences to replace the CDR sequences of the corresponding human antibody gene. The resultant antibody is entirely human except for the CDR (i.e. the antigen-binding region). The main advantage for this type of antibody is that the antibodies are significantly less immunogenic when compared with chimeric antibodies and it has a half-life approximately equal to fully-native human antibodies.

L. Production of humanized 3D6 antibodies

A preferred embodiment of the present invention is a humanized antibody to the N-terminus of amyloid-beta. 3D6 is a preferred starting material for the production of humanized antibodies in that it is specific for the N-terminus of amyloid-beta and it is capable of inducing phagocytosis of amyloid. A preferred humanized 3D6 antibody contains 1) a light chain comprising a variable domain comprising murine 3D6 variable light chain CDRs and a human acceptor framework having at least one, but preferably two to four residues selected from the group consisting of L1, L2, L36, and L46 substituted with the corresponding 3D6 residue and 2) a heavy chain comprising 3D6 variable heavy CDRs and a human acceptor framework having at least one, but preferably two or three residues selected from the group consisting of H49, H93, and H94 substituted with corresponding 3D6 residue and, optionally, at least one, but preferably two or three residues selected from the group consisting of H74, H77, and H89 substituted with a corresponding human germline residue.

In a more preferred embodiment, a humanized antibody of the present invention contains 1) a light chain comprising a variable domain comprising murine 3D6 variable light chain CDRs with a human acceptor framework having residue 1 substituted with a Tyr, residue 2 substituted with a Val, residue 36 substituted with a Leu, and residue 46 substituted with an Arg and 2) a heavy chain comprising 3D6 variable heavy chain CDRs with a human acceptor framework having residue 49 substituted with an Ala, residue 93 substituted with a Val, residue 94 substituted with an Arg and, optionally, residue 77 substituted with a Thr or residue 89 substituted with a Val.

In a preferred embodiment of the invention, the therapeutic agent exhibits at least one of the following functions: 1) binds aggregated amyloid-beta_{42} as determined using ELISA, 2) binds amyloid-beta as identified using the appropriate stains and microscopic methods, 3) binds amyloid-beta with two to three fold higher binding affinity as compared to a chimeric 3D6, 4) mediates phagocytosis in an ex vivo phagocytosis assay and 5) crosses the blood-retinal, blood-optic nerve, or blood-brain barrier.

Evidence that peripherally administered antibodies of interest gain access to the retina at levels sufficient to decorate and disaggregate amyloid-beta plaques can be obtained from clinical trials in patients with ARMD. The assay employed to evaluate the therapeutic agent will be standard fundus
photography by which one skilled in the art compares retinal photographs before treatment with those after treatment and quantitatively or qualitatively evaluates the number and confluence of the drusen.

M. Nucleic acid encoding immunologic and therapeutic agents

Immune responses against amyloid can 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 an enhancer, that allow expression of the DNA segment in the intended target cells of a patient. Expression in blood cells is desirable for induction of an immune response. To obtain expression in blood cells, 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.

In addition to antibodies, potentially any compound which binds specifically to amyloid-beta or modulates amyloid-beta may be a therapeutic agent within the context of the instant invention. In one embodiment of the invention, a peptide or nucleic acid aptamer, i.e. a nucleic acid molecule that binds to a specific ligand, of amyloid-beta can be used as a therapeutic agent, Osborne, S.E., et al., Curr. Opin. in Chem. Biol., 1:5-9 (1997) and Hoppe-Seyler, F. and Butz, K., J. Mol. Med., 78:426-430 (2000). Nucleic acid aptamers have been used in compositions by chemical bonding to a carrier molecule, Brody and Gold, Rev. Mol. Biol., 74:3-13 (2000). Peptide aptamers may be used in therapeutic applications by the introduction of an expression vector directing aptamer expression into the affected tissue such as, for example, by retroviral delivery, by encapsulating the DNA in a delivery complex or simply by naked DNA injection. In an alternative embodiment, the aptamer itself or a synthetic analog may be used directly as a drug, Hoppe-Seyler and Butz, supra.

A number of suitable viral vector systems are available, including, but not limited to, retroviral systems, adeno-viral vectors, viral vectors from the pox family, viral vectors from the alpha virus genus such as those derived from Sindbis and Semliki Forest viruses, Venezuelan equine encephalitis virus and rhabdoviruses such as vesicular stomatitis virus and papilloma viruses. In an alternative embodiment, vectors encoding immunogens can be delivered ex vivo to cells, such as, cells explanted from an individual patient’s lymphocytes, bone marrow aspirates, tissue biopsy, or universal donor hematopoietic stem cells.

Mouse studies using monoclonal antibodies

In vivo studies have been conducted with mice using the monoclonal antibodies 3D6 (WO 02/46237), 10D5 (WO 94/10569) and 16C11 (WO 0246237). After multiple months of treatment, 10D5 and 3D6 reduced plaque burden in the brain relative to control, however, 16C11 failed to have any effect on plaque burden. These results demonstrated that an antibody response against amyloid-beta peptide, in the absence of T cell immunity, is sufficient to decrease amyloid deposition in mice. Without wishing to be bound by any theory, it appears that antibodies directed to epitopes comprising N-terminal
a amino acids of amyloid beta are particularly efficacious. As a whole, these data suggest that the antibodies gained access to the CNS at levels sufficient to affect the clearance of amyloid plaques.

**Immunological approach to clearing amyloid-beta from the brain and possibly the sub-RPE space**

One embodiment of the invention uses an immunological approach to clearing amyloid-beta from the brain. Generally, an immunological approach involves either stimulating the host immune system to recognize and attack amyloid-beta actively or administering antibodies passively that reduce the amount of amyloid-beta present, prevent amyloid-beta from being deposited, or both. Active immunization may be carried out using either synthetic intact amyloid-beta_{42} or conjugated fragments thereof. Passive immunization may be carried out using an antibody to human amyloid-beta, Schenk, D., *Nature Reviews Neuroscience*, 3:824-828 (2002), an immunogenic fragment of an anti-amyloid beta antibody, or a nucleic acid molecule that is capable of inducing an immune response by specifically binding to a ligand, such as an aptamer, Osborne, S. E., *et al.*, *Curr. Opin. in Chem. Biol.*, 1:5-9 (1997).

A. Active immunization in mouse (murine) models

The PDAPP transgenic mouse over expresses amyloid precursor protein (APP). These mice develop many of the neuropathological hallmarks of Alzheimer's Disease (AD) in similar areas of the brain and in a similar age-dependent manner. In a study, one group of transgenic mice were immunized with amyloid beta at six weeks of age, which was before the onset of their AD-like neuropathology; while another group of PDAPP mice was immunized with amyloid beta at eleven months of age, i.e. after amyloid beta deposition and neuropathological changes had begun, Schenk, D., *et al.*, *Nature*, 400:173-177 (1999). Immunization of the young mice essentially prevented the development of amyloid beta plaques. Treatment of the older mice markedly reduced the extent and progression of these AD-like neuropathologies.

At eighteen months of age (and seven months after initiating immunizations at month eleven), the cortical amyloid-beta burden of immunized PDAPP mice was 0.01% vs. 4.87% for untreated age-matched controls, Schenk, D., *et al.*, 1999, *supra*. Thus, amyloid beta immunization cleared pre-existing amyloid beta plaque and prevented the development of new plaques. The data suggest that a highly specific immune response can markedly reduce pathology in PDAPP transgenic mice.

B. Passive immunization in mouse (murine) models

Experiments were conducted to test whether a humoral response alone, without a cellular response to amyloid beta, would be sufficient to clear plaques. Nine month old transgenic mice carrying a mutation for APP were injected intravenously with a mouse monoclonal antibody (mAb). The monoclonal antibodies used for these studies were 3D6, a monoclonal antibody specific for amyloid-beta_{1,5} (WO 02/46237), 10D5, a monoclonal antibody specific for amyloid-beta_{1,10} WO 94/10569 and 21F12, a monoclonal antibody specific for amyloid beta_{3,42} (U.S. Pat. No. 6,610,493).

Examination of brain sections showed that 3D6 and 10D5 entered the brain and partially bound to amyloid beta plaques. Subsequent experiments showed that 3D6 and 10D5 antibodies triggered microglial cells to clear amyloid-beta plaques through Fc receptor mediated phagocytosis and subsequent
degradation. No response was observed for the mice immunized with 21F12. Without wishing to be bound by any theory, it appears that antibodies against the N-terminal region of amyloid-beta are most associated with plaque clearance and that the epitope to which the antibody binds has some influence on the level of plaque clearance and neuroprotection. It further appears that high affinity of the antibody for Fc receptors on microglial cells has more influence on effect than high affinity for amyloid beta per se, Bard, F., et al., P.N.A.S., 100:2023-2028 (2003). Bard demonstrated that IgG2a antibodies have higher affinity compared with other isotypes for phagocytic Fc receptors (especially the surface receptor Fc-gamma-RI). IgG2a antibodies provided the highest level of plaque clearance and did show some neuroprotection. Activation of the phagocytic cells resulted in clearance of amyloid-beta as well as altering the inflammatory environment via the regulation of interleukins, Bard, F., et al., supra. Thus, clearance of amyloid beta plaques from the brain may involve two mechanisms that operate separately or in tandem: direct interaction of the antibodies with the amyloid beta resulting in a disaggregation, utilizing cell-mediated removal after a disaggregation event, or both Bacskai, B., et al., Nature Medicine, 7:369-372 (2001).

C. Clinical trials using an active immunization approach

Clinical trials were conducted using AN-1792, which is a synthetic amyloid-beta_{42} in combination with adjuvant QS-21 (Elan Pharmaceuticals/Wyeth). Phase I consisted of two dose-escalating studies with about 1 to 3 months prior to dose escalation to the next level. AN-1792 was well tolerated.

Phase IIa trials were conducted with 375 AD patients with 300 patients receiving multiple doses of the highest dose of amyloid-beta_{42} combined with the lowest dose of QS-21. Treatment was suspended in this trial after 17 AD patients (17/300 or 6%) developed signs and symptoms consistent with meningoencephalitis. Each of these 17 patients had received between one to three doses of AN 1792 Schenk, D., Nature Reviews | Neuroscience 3:824-828 (2002). The duration and severity of the symptoms varied considerably. The signs consisted of transient changes on MRI scans and elevated white counts in the CSF, indicating central inflammation. Although dosing was terminated, coding was maintained unbroken; MRI scans and cognitive studies continued into 2003. Post mortems conducted on tissue samples subsequent to ending the clinical trial demonstrated that active immunization approach was able to clear plaques from the brain. See, Hensley, S., New Hope Seen for Vaccine for Alzheimer’s, Wall Street Journal, July 22, 2004; Nicoll, J. A., Nature Medicine 9:448-452 (2003).

D. Theoretical advantages of passive immunization compared with active immunization

Based on the published date, passive immunization appears to have advantages versus active immunization including, but not limited to, that it would be self-limiting, may have the optimal epitope characteristics, and appears to avoid the need for an elderly person to mount a robust immune response, Bacskai, B., et al., supra.
Treatment regimens

The present invention includes the administration of therapeutic immunological reagents such as humanized immunoglobulins to specific epitopes within amyloid-beta to a patient that will benefit and show a beneficial therapeutic response such as, but not limited to, induction of phagocytosis of amyloid-beta, reduction of plaque burden, inhibition of plaque formation, and/or reversing, treating, or preventing visual disability. The invention is also directed to the use of the disclosed immunological reagents in the manufacture of a medicament for the treatment or prevention of an amyloidogenic disease of the eye or visual system.

The term “treatment” as used herein describes the in vivo administration of a therapeutic agent to a patient, or in vitro or ex vivo administration of a therapeutic agent to an isolated tissue or cell line from a patient, who has a disease, symptom of a disease or predisposition toward a disease, with the purpose to cure, alleviate, heal, relieve, alter, remedy, ameliorate, improve or otherwise affect the disease, symptoms of the disease or the predisposition toward the disease.

Medicaments related to the invention may be administered to a patient with an amyloid beta visual disability in one or both eyes or to a patient at risk for developing visual loss secondary to either non-exudative ARMD (including geographic atrophy), exudative ARMD, or glaucoma. An effective treatment within the scope of this invention will provide symptom relief for those experiencing a related disease, will arrest the progression of the disease state in patients with active disease, or will delay the onset of the disease in patients who are at risk but who do not have active disease, especially those who have ARMD in one eye and are at risk for developing ARMD in the second eye.

A. Dosage regimens

Effective doses of the compositions of the invention for therapeutic treatments will vary and those of ordinary skill in the art will know how to adjust the dosage taking into consideration factors that include, but are not limited to, the means of administration, route of administration, target site, physical condition of the patient, and whether the treatment is intended to be for therapeutic or prophylactic purposes.

For passive immunization, the dosage per administration can range from about 0.0001 to 100 mg/kg. Preferably dosages range from about 0.01 to 5 mg/kg, more preferably from about 1 mg/kg to 10 mg/kg and most preferably about 1 mg/kg of body weight are utilized.

Exemplary dosage schedules may include 1 to 10 mg/kg on consecutive days, 30 mg/kg on alternate days or 60 mg/kg weekly. The therapeutic agent may be administered as a sustained release formulation. Treatment regimes within the scope of the invention can be individually adjusted such that the administered dosages achieve a plasma concentration of 1 to 1,000 µg/mL and more preferably from about 25 to 300 µg/mL. Those of ordinary skill in the art will understand that if the therapeutic agent is to be administered as a sustained release formulation, less frequent administration will be required.

An effective prophylactic dose may vary based upon the patient’s general state of health and immunity, typically dosages range from 0.1 to 25 mg per dose and more preferably from about 0.5 to 2.5 mg per dose. The dose would be administered relatively infrequently over a long time interval.
Compositions of the invention are preferentially prepared and administered as injectables, either as liquid solutions or suspensions. However, solid forms, including lyophilized forms suitable for solution or preparation as a suspension in a liquid, may also be administered. Compositions of the invention may also be emulsified or encapsulated in liposomes or micro particles such as polylactide, polyglycolide, or copolymer for enhanced adjuvant effect. Agents of this invention can also be formulated to permit sustained or pulsatile release of active ingredient.

The progress of a treatment can be monitored by assaying antibody levels over time relative to a control or base level or by using standard clinical methods of assessing disease progression such as retinal photography and retinal angiography. These methods are known to those of ordinary skill in the art. See, Tyler, M., et al., Practical Retinal Photography and Digital Imaging Techniques, Elsevier (2003). Repeated cycles of the therapeutic agents of the invention can be administered when circulating antibody titers decline or when clinical symptoms of the disease becomes manifest. Therapeutic agents of the invention can be administered daily, weekly, monthly, annually or at irregular intervals, i.e. on an as needed basis. In some embodiments, two or more therapeutic agents with differing binding specificities may be administered simultaneously.

Dosage and frequency will vary depending on the half-life of the therapeutic agent administered to the patient. Generally, the half-life of the human antibodies is longer than for humanized, chimeric and non-human antibodies.

Dosage and frequency of administration can vary depending on whether the treatment is for therapeutic or prophylactic purposes. For therapeutic use, a relatively high dosage is given at relatively short intervals until the progression of the disease is reduced or terminated. Thereafter, the patient can be administered a prophylactic regimen. For prophylactic use, a relatively low dosage is administered at relatively infrequent intervals over a long time. Some patients will continue to receive treatment for the rest of their lives.

In another aspect of this invention, the therapeutic agent is 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. Dosages for nucleic acids encoding an antibody generally range from about 10 ng to 1 g, preferably from about 100 ng to 100 mg, more preferably from about 1 μg to 10 mg, and most preferably from about 30 to 300 μg DNA per patient. Doses for infectious viral vectors vary from 10 to 100 or more virions per dose.

B. Routes of administration

For administration of antibodies, intravenous infusion and intramuscular injection are preferred. Other routes of administration may include, but are not limited to, subcutaneous, transdermal (including the use of transfersomes), intradermal, oral, intranasal, topical, intracerebral, intracameral, intravitreal, sub-Tenons or combinations thereof. Pulmonary and suppository formulations are other possible routes. Oral formulations may take the form of solutions, suspensions, tablets, pills, capsules, and sustained release formulations.
Intravenous infusion or intramuscular injection are the preferred methods for the administration of antibodies. Antibodies can be administered in the form of a depot injection or implant preparation, which can be formulated for sustained release of the active ingredient. An exemplary composition comprises a monoclonal antibody at about 5 mg/mL, formulated in an aqueous buffer consisting of 50 mM L-histidine, 150 mM NaCl, adjusted to pH 6.0 with HCL. Implants can also be formulated for pulsatile release of the antibodies.

The diluent is selected so as not to affect the biological activity of the combination. Other examples of diluents include distilled water, physiological phosphate-buffered saline, Ringer’s solutions, dextrose solution, and Hank’s solution. Pharmaceutical formulation can also include other carriers, adjuvants, or nontoxic, non-therapeutic, nonimmunogenic stabilizers.

Compositions of the invention may be prepared as injectables, either as liquid solutions or suspensions. Pharmaceutical compositions can also include large, slowly metabolized macromolecules such as proteins, polysaccharides such as chitosan, polyacetic acids, polyglycolic acids, polymeric amino acids, amino acid copolymers, lipid aggregates such as oil droplets or liposomes, or copolymers such as latex functionalized sepharose™, agarose, and cellulose.

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, oil, saline, ethanol, or glycerol. Auxiliary substances such as wetting or emulsifying agents, surfactants, and pH buffering substances can be present in compositions. Other components of pharmaceutical compositions include: petroleum, 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.

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

Topical administration can be facilitated by co-administration of the agent with cholera toxin or detoxified derivatives or subunits thereof or other similar bacterial toxin, Glenn, Nature, 391:851 (1998). Co-administration can be achieved by using the components as a mixture or as linked molecules obtained by chemical cross-linking or expression as a fusion protein.

C. Time-course of passive immunization and repeated cycles of treatment

Following administration of the antibodies, the antibody concentration in whole blood, blood plasma, or blood serum will show an immediate peak followed by an exponential decay. Without additional dosing, the decay may approach pretreatment levels within a period of days to months, depending on the half-life of the antibody. The half life of some human antibodies is about twenty days.

D. Alternative body fluids include tear fluid, saliva, and mucous.

In some embodiments of the invention, a baseline measurement of antibody to amyloid-beta is made prior to administration and a second measurement is made soon thereafter to determine the
peak level. Additional measurements are made at intervals to monitor decay of antibody levels. When antibody concentration has declined to the baseline level or a predetermined percentage of the peak (e.g. 50%, 25%, or 10%) has decreased, repeated dosing may be warranted. In still other embodiments, peak levels or subsequently measured concentrations are compared with a predetermined reference concentration, i.e. control, to evaluate the prophylactic or therapeutic treatment regimen. If measured antibody concentration is significantly less than a reference value, administration of a repeated dose is indicated.

E. Treatment with concomitant medications

The present invention may be used with other therapeutic agents, outside the scope of this invention, that are concomitantly or sequentially administered to enhance their pharmacologic effect. The term “concomitantly administered” as used herein means to administer the agents at essentially the same time or substantially concurrently. The term “concomitantly administered” encompasses not only administration of the two agents in a single pharmaceutical dosage formulation, but also the administration of each active agent in its own separate pharmaceutical dosage formulation. The term “sequentially administered” means to administer the agents at separate and staggered times. The agents can be sequentially administered such that the beneficial pharmaceutical effect of each agent is realized by the patient at the same time.

The following are co-therapy agents that may be considered to enhance the pharmacologic effect of the compositions within the scope of this invention.

1. Anti-inflammatory agents may be used with the present invention to reduce the inflammation caused by amyloid-beta assemblies in the sub-retinal space or within the optic nerve or by other inflammatory entities within the sub-retinal space or within the optic nerve. Examples of such anti-inflammatory agents include cyclooxygenase-2 (COX-2) inhibitors, such as topical (rofecoxib) and oral (e.g., VIOXX (rofecoxib), CELEBREX (celecoxib), BEXTRA (valdecoxib); nonsteroidal anti-inflammatory agents (NSAIDS) both oral and topical (e.g., diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, ketorolac, meclofenamate, mefenamic acid, meloxicam, nabumetone, naproxen, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin); Corticosteroids both topical and oral (e.g., dexamethasone, fluorometholone, hydrocortisone, loteprednol, medrysone, prednisone, prednisolone, rimexolone); aspirin; and immunosuppressants such as Restasis (cyclosporine A).

2. Anti-angiogenic agents for the inhibition of neovascularization, such as Lucentis™ (ranibizumab), Macugen™ (pegaptanib), Retanene™ (anecortave) and photodynamic therapies.

3. Small Interfering RNA molecules (siRNA), short sequences of double-stranded RNA which suppress expression of target genes, that can be used to silence and regulate genes associated with angiogenic disease of the eye or optic nerve, for example, vascular endothelial growth factor (VEGF), a protein implicated with macular degeneration and diabetic retinopathy.

4. Tyrosine kinase regulators, such as pyrazolo [1, 5-a] pyrimidine derivatives disclosed in WO 04/052286 and WO 04/052315.
5. Anti-oxidants for the protection of RPE cells against oxidative stress, such as a isothiocyanate sulforaphane or a bis-2-hydroxybenzylideneacetone, a Michael reaction acceptor, or other known anti-oxidants such as melatonin, vitamin E, vitamin C, zinc, non-selective beta-blockers such as carvedilol, OH-carvedilol, timolol, levobunolol, and carteolol.

6. N-methyl-D-aspartate (NMDA) antagonists that have a neuroprotective effect, thereby reducing excitotoxic damage of retinal neurons, such as memantine (Nemenda®), cerestat (Cambridge Neuroscience CNS 1102) and rifuzole (Rilutek®).

7. HMG-coA reductase inhibitors for aortic stenosis, such as atorvastatin (Lipitor®), fluvastatin (Lesco®), lovastatin (Mevacor®), pravastatin (Pravachol®), rosuvastatin (Crestor®) and simvastatin (Zocor®).

8. Carbonic anhydrase inhibitors to increase peripapillary choroidal perfusion in non-exudative AMRD patients, such as dorzolamide (Trusopt®) ophthalmic solution and Brinzolamide (Azopt®) ophthalmic suspension.

9. Integrin antagonists for the survival and maturation of newly formed but not quiescent retinal blood vessels, such as vitronectin receptor antagonists.

10. Metalloproteinase inhibitors for maintaining tissue integrity and remodeling, as in wound healing, processes of development, and regeneration.

11. Neurotrophic factors, such as neuropoietic cytokines, for example, ciliary neurotrophic factor (CNTF), neuropoietic cytokines, neurotrophins, fibroblast growth factors, transforming growth factors, glial cell-line derived neurotrophic factor, neurotrophin 3, neurotrophin 4/5, and interleukin 1-beta, and pigment epithelium-derived factor (PEDF).

All of these listed agents can be co-administered in forms compatible with the therapeutic agents of the invention herein and are to be administered in the manner and dosage normally associated with the agent.

Other agents that can be co-administered with the therapeutic agents of the invention herein include, but are not limited to, secretase inhibitors (beta and gamma), caspase inhibitors, stem cells, hyaluronidase, mitotic kinesis inhibitors, alkyl sulfonyl pyrazoles, maxi-K channel antagonists and other antibodies or fragments of antibodies directed towards the clearance of amyloid-beta peptide, e.g., IgG1 (10d5) or IgG2b (3D6). Medications that activate or promote the development of ApoE (apolipoprotein E) ε4 allele or suppress ApoE ε2 or ε3 alleles, compounds that can block activity or development of immature amyloid development and deposition, or compounds which can block, interpret or disassemble the transthyretin (TTR) protein, singularly or in aggregate form, with an individual or aggregate weight of fibril or protein aggregate less than or equal to 100 kDa may also be administered with the compounds of the invention.

Still other agents that can be co-administered with the therapeutic agents herein may include factors that prevent the growth of new blood vessels, promote vessel maturation, inhibit permeability of blood vessels, inhibit migration of endothelial cells, such as the anti-angiogenic factors described in WO 02/22176, pigment epithelium-derived factor, angiostatin, vasculostatin, endostatin,
platelet factor 4, heparinase, interferon, tissue inhibitor metalloproteinase 3, antisense molecules specific for an angiogenic factor and phosphorothiotic oligodeoxynucleotides. One with ordinary skill in the art will understand that any of these co-agents can be modified and administered for use with the present invention.

The following examples are to illustrate the invention and should not be interpreted as any limitation on the scope of the invention herein.

EXAMPLES

1. Screening antibodies for binding to the amyloid beta in the drusen of patients with ARMD

   The therapeutic agents of the invention are intended to clear the amyloid component in drusen associated with ophthalmic diseases. Selection of the appropriate therapeutic agent is carried out by screening for antibodies or other immunogenic agents that have the desired binding characteristics. To screen for therapeutic agents that actively bind the amyloid beta deposit within drusen, a post-mortem tissue sample from the retina of a patient or animal with amyloidogenic disease is contacted with the Fab component(s) of the monoclonal antibody being tested in vitro. In some embodiments, the components are combined on a microscopic slide to facilitate microscopic monitoring.

   It would be desirable in screening for such agents to perform multiple reactions in parallel in the wells of a microtiter dish. A standard enzyme-linked immunosorbent assay (ELISA) assay can be used to carry out the selection of the therapeutic agent in which a primary antibody binds to the protein in question (antigen) and is in turn recognized by a secondary antibody, which is linked to an indicator enzyme whose activity can be quantified. The activity measured is directly proportional to the amount of primary antibody and, consequently, of the antigen. Preferably, a series of measurements is made of the amount of amyloid deposition in the in vitro reaction mixture, beginning with a baseline or control value before the addition of the secondary antibody and continuing with additional test values collected during the reaction. The antigen can be detected by fluorescent staining. An antibody can be labeled with fluorescent stain to amyloid-beta or other components of amyloid. The antibody used for staining may or may not be the same antibody being tested for amyloid-beta clearing activity. A reduction relative to a baseline level indicates that the antibody being evaluated has the desired amyloid-beta clearing activity. Such antibodies are likely to be useful in treating or preventing the progression of amyloidogenic diseases of the eye or visual pathways.

2. Patients amenable to treatment

   Patients amenable to treatment include patients who currently have a visual disability in one or both eyes related to the presence of amyloid-beta in the retina or optic nerve. Patients amenable to treatment also include patients who currently do not have a visual disability in either eye related to the presence of amyloid-beta in the retina or optic nerve but have been identified having factors placing them at risk. Thus, the present invention can be administered prophylactically as well as therapeutically. The object of the invention is the administration of therapeutic agents that are capable of lessening the severity
or delaying the onset of the amyloidogenic disease or reducing the risk that such a disease will progress. Patients that are amenable to treatment with the compounds of the invention include those individuals who have been identified in one or both eyes using standard ophthalmological diagnostic methods, clinically-observed markers for amyloidogenic diseases as well as those identified having indirect markers for such diseases. The present invention will be especially useful in patients who have developed opthalmic disease asymmetrically, i.e. one eye is affected while the second eye appears normal using clinical methods.

3. Treatment of amyloidogenic disease of the eye or retina

A. Screening for retinal drusen

To determine whether the therapeutic agent screened above would clear or disaggregate amyloid found within retinal drusen of post-mortem eyes of patients diagnosed with Geographic Atrophy (GA) or a combination of GA and exudative ARMD, post-mortem eyes will be obtained from an eye bank, such as the Population-based Eye Pathology: Service Laboratory, an Age-related Maculopathy Histopathology Laboratory, Birmingham, AL or a similar laboratory that provides human eye tissue. Tissue specimens of the post-mortem eyes will be obtained from at least one eye, and preferably two eyes, per donor. Following dissection of the anterior segment, eyecups will be fixed using freshly prepared 4% paraformaldehyde in 0.1 M sodium cacodylate buffer (pH 7.4). After overnight fixation, the eyecups will be stored in a 0.4% solution of the same fixative. At least one eye from each donor is evaluated for the presence of drusen and amyloid-beta assemblies. Ninety micrometer thick vibratome sections of paraformaldehyde-fixed, hydrated RPE/choroidal tissue are examined using differential interference contrast optics. Color photographs are taken using a digital camera or digitized from conventional color reversal film and analyzed for the presence of drusen and/or amyloid-beta assemblies in the sample.

B. Amyloid histochemistry

Sections containing drusen and/or amyloid-beta assemblies are stained using Thioflavin T (Sigma Chemical; St. Louis, MO, USA) using established protocols, Mullins, R., et al., FASEB J., 14:835-846 (2000). Thioflavin T is a non-specific stain for amyloid fibrils with a beta-sheet structure. Stained samples are examined using ultraviolet fluorescence optics (Olympus UG-1 filter set). Human liver and cornea section samples are used as positive control tissue for amyloid staining.

C. Laser scanning confocal microscopy

Multiple specimens of RPE-choroidal tissue from one or both eyes are examined using confocal immunofluorescence microscopy. Specimens about 4 mm in diameter are obtained from the posterior pole and near periphery using established protocols, Anderson, D., et al., Am J. Ophthalmol., 131:767-781 (2001). Vibratome sections of agarose embedded tissue will be blocked with normal donkey serum in 100 mM sodium phosphate buffer (pH 7.1) containing 0.5% bovine serum albumin (Fraction V, Sigma), 0.05% Triton X-100 and 0.1% sodium azide. The sections are exposed to the therapeutic agents, which include 3D6, 10D5, 21F12, 6E10, 4G8 and 22C11, in the same buffer. Incubation of the treated
samples is followed by rinsing and overnight incubation in species-specific donkey secondary antibodies conjugated with indocarbocyanin-2, -3, or -5. Untreated samples are used as controls for non-specific labeling, including the omission of primary antibody.

Fluorescent probes that bind to amyloid such as Thioflavin T are known to those of skill in the art, Anderson, D., et al., Exp. Eye Res., 78:243-256 (2004). The probes are applied to vibratome sections at stock dilutions ranging from 1:1000 to 1:3000 for one hour in phosphate buffered saline (PBS) and then washed in PBS. Triton X-100 is omitted at times from the buffer to minimize extraction of components caused by exposure to detergent.

After processing, sections are mounted on glass slides in glycerol containing 1% (w/v) N-propyl-gallate as an anti-quenching agent and examined using a laser scanning confocal microscope. Digital images are acquired. It may be desirable in some instances to visualize the autofluorescence emitted by lipofuscin pigment in RPE cells and Bruch’s Membrane by optimizing the gain on the red channel.

D. Immunogold electron microscopy

Paraformaldehyde fixed tissue specimens are dehydrated through a graded ethanol (ETOH) dilution series up to 100%, infiltrated in a 1:1 ETOH/LR White mixture overnight, and then embedded in 100% LR White acrylic resin at 60 degrees centigrade. Blocks are sectioned at 75 nm in thickness and sections are placed on parlodian-coated nickel grids. For immunostaining, sections on grids are etched with 0.5% sodium metaperiodate for 10 minutes at room temperature, and then washed (5X at 10 minutes per wash) on drops of 0.025 M Tris buffer (pH 7.4). Sections are pre-incubated in a blocking solution of 5% bovine serum albumin in 0.025 M Tris buffer for 15 minutes at room temperature, and then incubated for two hours at 37 degrees centigrade with at least one therapeutic agent, including the aforementioned amyloid beta antibodies. After incubation, the grids are washed in 0.025 M Tris buffer and blotted on filter paper. After rinsing, the grids are incubated in 0.025 M Tris buffer containing a 1:250 dilution of goat anti-mouse IgG conjugated at 10 nm colloidal gold at 37 degrees centigrade for 45 minutes, and then rinsed repeatedly in buffer and 2.25 M NaCl. After a final rinse in buffer, the grids are immersed in double distilled 0.2 micrometers filtered water to prevent salt accumulation on the grid surface. The grids are then stained with saturated uranyl acetate and lead citrate and then visualized in transmission electron microscopy.

Therapeutic agents within the scope of this invention are agents that demonstrate the ability to bind in a visually observable manner the amyloid beta of retinal drusen in a manner similar to the way in which monoclonal antibodies 3D6 and/or 10D5 decorate the plaques in brains of AD patients.

Although the foregoing invention has been described in detail for purposes of clarity and understanding, those skilled in the art understand that certain modifications may be practiced within the scope of the claims herein. All publications and patents cited herein are incorporated by reference in their entirety as if each were set forth at length herein.
WHAT IS CLAIMED:

1. A method for prophylactically or therapeutically treating an ophthalmic disease characterized by amyloid deposition in the eye or optic nerve comprising the administration of an effective dose to a patient in need thereof of at least one therapeutic agent that binds to a component of said amyloid deposits.

2. The method of claim 1 wherein said ophthalmic disease is Age-Related Maculopathy (ARM), non-exudative Age-Related Macular Degeneration (ARMD), exudative ARMD, or Glaucomatous Optic Neuropathy (GON).

3. The method of claim 1 wherein said patient has a perceived clinical risk factor or factor(s) or inherited risk factor(s) for the development or progression of ARM, ARMD, or GON.

4. The method of claim 1 wherein the patient has no known clinical risk factor or factor(s) or inherited risk factor(s) for the development or progression of ARM, ARMD, or GON.

5. The method of claim 1 wherein said component of an amyloid deposit is amyloid beta.

6. The method of claim 1 wherein the therapeutic agent is a monoclonal antibody to amyloid beta.

7. The method of claim 6 wherein the therapeutic agent is a humanized antibody.

8. The method of claim 7 wherein the antibody specifically binds to an epitope comprising a free N-terminal residue of amyloid beta.

9. The method of claim 7 wherein the antibody specifically binds to an epitope within residues 1-10 of amyloid-beta.

10. The method of claim 1 wherein the therapeutic agent is administered intravenously, intramuscularly, subcutaneously, topically, transdermally, intradermally, orally, intranasally, intravitreally, intrasclerally, juxtasclerally or intracamerally.

11. The method of claim 1 wherein the level of the therapeutic agent is measured in a body fluid as an antibody titer or using an ELISA assay.
12. The method of claim 1 wherein the therapeutic agent specifically binds to a component of said amyloid deposit in the patient within the retina or optic nerve and induces a clearing response against the amyloid deposit.

13. A method for prophylactically or therapeutically treating an ophthalmic disease characterized by amyloid deposition in the eye or optic nerve comprising the administration of an effective dose to the patient of at least one therapeutic agent that induces an immune response to said amyloid deposit.

14. The method of claim 1 wherein the therapeutic agent induces an immune response to amyloid-beta.

15. The method of claim 1 wherein the therapeutic agent is a monoclonal antibody to amyloid-beta.

16. The method of claim 15 wherein the therapeutic agent is a humanized antibody.

17. The method of claim 16 wherein the antibody specifically binds to an epitope comprising a free N-terminal residue of amyloid-beta.

18. The method of claim 16 wherein the antibody specifically binds to an epitope within residues 1-10 of amyloid-beta.

19. The method of claim 13 wherein the therapeutic agent is administered intravenously, intramuscularly, subcutaneously, topically, transdermally, intradermally, orally, intranasally, intravitreally, intrasclerally, juxtasclerally or intracameraly.

20. The method of claim 13 wherein the level of the therapeutic agent is measured in a body fluid as an antibody titer or using an ELISA assay.

21. The method of claim 13 wherein the therapeutic agent specifically binds to a component of said amyloid deposit in the patient within the retina or optic nerve and induces an immune response to said amyloid deposit.
SEQUENCE LISTING

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Methods of Treatment or Prophylaxis of Amyloidogenic Diseases of the Eye or Optic Nerve

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