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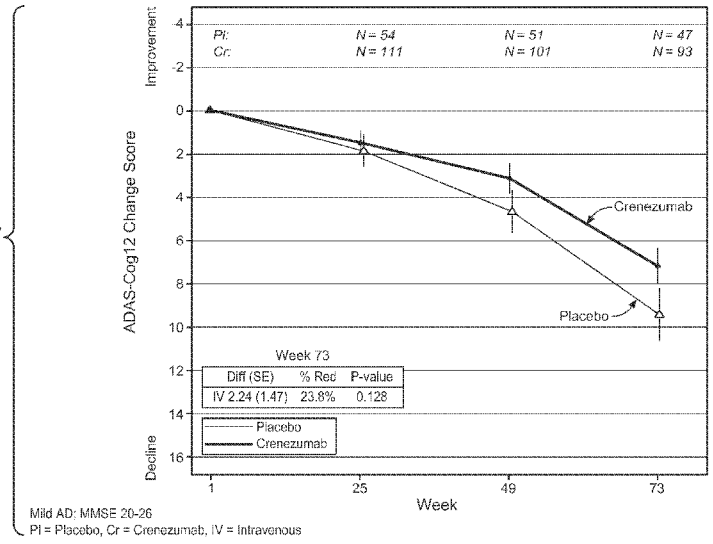
*[Continued on next page]*

(54) Title: METHODS OF TREATING ALZHEIMER'S DISEASE

FIG. 7




(57) Abstract: Methods of treating Alzheimer's Disease (AD) in patients suffering from mild to moderate AD, including ApoE4 positive patients and patients suffering from mild AD are provided.

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# METHODS OF TREATING ALZHEIMER'S DISEASE

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims the benefit of U.S. Provisional Application No. 61/937,472, filed on February 8, 2014, U.S. Provisional Application No. 61/971,479, filed on March 27, 2014, U.S. Provisional Application No. 62/010,259, filed on June 10, 2014, and U.S. Provisional Application No. 62/081,992, filed on November 19, 2014, the contents of the foregoing applications are incorporated herein by reference in their entirety.

## FIELD

**[0002]** Methods of treating patients suffering from mild to moderate Alzheimer's Disease using antibodies that target amyloid β are provided.

## BACKGROUND

**[0003]** Alzheimer's Disease (AD) is the most common cause of dementia, affecting an estimated 4.5 million individuals in the United States and 26.6 million worldwide (Hebert et al, Arch. Neurol. 2003; 60:1119-22; Brookmeyer *et al.*, Alzheimers Dement. 2007; 3:186-91). The disease is characterized pathologically by the accumulation of extracellular β-amyloid ("Aβ") plaques and intracellular neurofibrillary tangles in the brain. Diagnosis is made through the clinical assessment of the neurologic and neuropsychiatric signs and symptoms of AD and the exclusion of other causes of dementia. AD is commonly classified into mild, moderate and severe stages by a brief cognitive screening examination, the Mini-Mental State Examination ("MMSE"). Approved medical therapies that inhibit acetylcholinesterase ("AChE") activity or antagonize N-methyl-D-aspartate receptors in the brain may temporarily improve the symptoms of AD in some patients but do not modify the progression of the disease (Cummings, N. Engl. J. Med. 2004; 351:56-67).

**[0004]** Genetic factors in early- and late-onset familial AD are now well documented. The ApoE4 allele is strongly associated with late-onset familial and sporadic AD, with a reported allele frequency of 50%-65% in patients with AD, which is approximately three times that in the general population and for other neurologic disorders (Saunders *et al.*, Neurology 1993; 43:1467-72,; Prekumar *et al.*, Am. J. Pathol. 1996; 148:2083-95). In addition to AD, the ApoE4 allele has been implicated in other amyloid-forming disorders, including cerebral amyloid angiopathy ("CAA") (Prekumar *et al.*, Am. J. Pathol. 1996;

148:2083-95). Thus, patients who carry the ApoE4 allele may represent an etiologically distinct population of patients with AD.

**[0005]** The deposition of extracellular amyloid plaques in the brain is a hallmark pathologic finding in AD, first reported by Alois Alzheimer in 1906. These amyloid plaques are primarily composed of Abeta peptides (Haass and Selkoe, Nature 2007; 8:656-67) generated by the sequential cleavage of amyloid precursor protein ("APP") via β and γ-secretase activity. Abeta, particularly in its oligomerized forms, is toxic to neurons and is believed to be causative in AD. Therapies that reduce Abeta levels in the brain may alleviate cognitive dysfunction and block further synaptic loss, axon degeneration, and neuronal cell death. Abeta can be transported actively across the blood-brain barrier (Deane *et al.*, Stroke 2004; 35(Suppl I):2628-31). In murine models of AD, systemic delivery of antibodies to Abeta increases Abeta levels in plasma while reducing levels in the central nervous system (CNS) through several proposed mechanisms, including dissolution of brain Abeta plaque, phagocytic removal of opsonized Abeta, and finally via efflux of Abeta from the brain as a result of an equilibrium shift of Abeta resulting from circulating antibodies (Morgan, Neurodegener. Dis. 2005; 2:261-6).

**[0006]** Significant failures have marked the development of therapeutic antibodies for the treatment of AD. Large-scale phase three clinical trials of bapineuzumab, an antibody binding specifically to the N-terminal portion of Abeta, were halted when administration of the drug failed to arrest cognitive decline in treated patients (Miles *et al.*, Scientific Reports 2013; 3:1-4 Johnston & Johnson press release dated August 6, 2012, entitled "Johnson & Johnson Announces Discontinuation of Phase 3 Development of Bapineuzumab Intravenous (IV) in Mild-To-Moderate Alzheimer's Disease"). Notably, bapineuzumab did appear to stabilize plaque levels and decreased phosphorylated tau levels in cerebrospinal fluid – suggesting that modification of these biomarkers alone is not necessarily predictive of clinical efficacy (Miles *et al.*, Scientific Reports 2013; 3:1-4). Similarly, in phase three clinical trials of solanezumab, an antibody specific for monomeric Abeta that binds in the middle portion of the peptide, the primary cognitive and functional endpoints were not met (Eli Lilly and Company press release dated August 24, 2012, "Eli Lilly and Company Aanounces Top-Line Results on Solanezumab Phase 3 Clinical Trials in Patients with Alzheimer's Disease"). Safety concerns have also been raised during the investigation of certain immunotherapies for AD; for example, incidence of amyloid-related imaging abnormalities (ARIA-E and ARIA-H) was over 20% among drug-treated patients in phase two clinical trials of bapineuzumab

(Sperling *et al.*, The Lancet 2012; 11:241-249). It is estimated that one in nine people over the age of 65 have AD -- the aggregated yearly costs for health care, long-term care and hospice care by and on behalf of individuals afflicted with AD are over $200 billion in 2013, and are estimated to rise to $1.2 trillion by 2050 (by and on behalf of affected individuals) (Alzheimer's Association 2013 Alzheimer's Disease Facts and Figures, Alzheimer's and Dementia 9:2). AD is the sixth-leading cause of death in the United States as of 2013 (*id.*). Current approved therapies treat only some of the symptoms of AD, and not the underlying degeneration. There is a tremendous unmet need for a disease-modifying therapeutic for AD.

## SUMMARY

**[0007]** Crenezumab (also known as MABT5102A) is a fully humanized IgG4 monoclonal antibody to Abeta selected for its ability to bind both monomeric and oligomeric forms of Abeta *in vitro*. Crenezumab binds both Abeta1-40 and Abeta 1-42, inhibits Abeta aggregation, and promotes Abeta disaggregation. Because crenezumab is a human IgG4 backbone antibody, it has reduced Fcγ receptor ("FcλR") binding affinity compared with human IgG1 or IgG2, which is predictive of reduced immune effector response. These properties, combined with the ability of systemically delivered crenezumab to decrease Abeta CNS levels in a murine model of AD, have suggested that this anti-Abeta therapeutic approach may offer clinical efficacy while reducing risk of toxicity, and might potentially be able to modify the disease progression of AD with lower risk of the potentially deleterious side effects, such as cerebral vasogenic edema or hemorrhages, which had previously been seen in the clinical trials of other Abeta antibody therapies.

**[0008]** The results of phase two clinical studies in AD patients described herein demonstrate that crenezumab indeed slows the progression of disease in mild to moderate AD, has an even stronger effect in ApoE4 positive patients and in patients suffering from mild AD, and shows the greatest therapeutic benefit in patients with the mildest AD. Furthermore, the effect is seen in patients having a brain amyloid load that is typically seen in patients diagnosed with AD. Additionally, the results demonstrate that these effects occur without significant incidence of adverse events such as ARIA-E and ARIA-H. This application thus provides methods for treating and monitoring patients diagnosed with mild to moderate AD, especially mild AD, and ApoE4 positive patients, as well as patients having brain amyloid accumulation that is typically seen in patients diagnosed with AD. As exemplified herein, it has now been discovered that a humanized monoclonal anti-amyloid

beta antibody with a conformational epitope specific for the middle region of amyloid beta (Aβ) peptide (*i.e.*, within amino acids 13-24, such as crenezumab) is effective to treat mild to moderate AD, especially ApoE4 positive patients and patients with milder forms of AD, such as, but not limited to, mild AD, without an increased incidence of ARIA-E or ARIA-H. Accordingly, this application provides therapeutic agents for modulating the severity of AD and improved methods of using the same.

**[0009]** Consequently, the present application provides methods of treating patients suffering from AD and other amyloidoses, comprising administering a humanized monoclonal anti-amyloid beta (Aβ or Abeta) antibody, or antigen-binding fragment thereof, that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1). In some embodiments, the antibody, or antigen-binding fragment thereof, is capable of binding fibrillar, oligomeric, and monomeric forms of Abeta. In some embodiments, the antibody is an IgG4 antibody. In particular embodiments, the antibody, or antigen-binding fragment thereof, comprises six hypervariable regions (HVRs) wherein HVR-H1 is SEQ ID NO:2, HVR-H2 is SEQ ID NO:3, HVR-H3 is SEQ ID NO:4, HVR-L1 is SEQ ID NO:6, HVR-L2 is SEQ ID NO:7, and HVR-L3 is SEQ ID NO:8. In some embodiments, the antibody, or antigen-binding fragment thereof, comprises a heavy chain having the amino acid sequence of SEQ ID NO:5, comprising a heavy chain variable region, and a light chain having the amino acid sequence of SEQ ID NO:9, comprising a light chain variable region. In a specific example, the antibody is crenezumab.

**[0010]** The methods of treatment provided herein can be applied to patients suffering from AD or other amyloidosis, as described further herein. Suitable patients include patients suffering from mild to moderate AD, patients with an MMSE score of 18 to 26, patients suffering from mild AD, patients with an MMSE score of 20 or above (*e.g.*, 20-30, 20-26, 24-30, 21-26, 22-26, 22-28, 23-26, 24-26, or 25-26), patients suffering from early AD (including patients having mild cognitive impairement due to AD and patients having preclinical AD), amyloid positive patients (or patients having brain amyloid load consistent with that seen in patients diagnosed with AD), and ApoE4 positive patients suffering from mild to moderate or mild AD.

**[0011]** In some aspects, the methods provided herein are methods of reducing decline due to AD in patients suffering from early, mild, or mild to moderate AD. In some embodiments, the decline is one or more of: clinical decline, cognitive decline, and functional decline. In some embodiments, the decline is clinical decline. In some embodiments, the decline is a

decline in cognitive capacity or cognitive decline. In some embodiments, the decline comprises a decline in functional capacity or functional decline. Various tests and scales have been developed to measure cognitive capacity (including memory) and/or function. In various embodiments, one or more test is used to measure clinical, functional, or cognitive decline. A standard measurement of cognitive capacity is the Alzheimer's Disease Assessment Scale Cognitive (ADAS-Cog) test, for example, the 12-item ADAS-Cog or ADAS-Cog12. Thus, in some embodiments, the reduction or slowing in decline in cognitive capacity (or cognitive decline) in patients being treated with the antibodies of the invention is determined using the ADAS-Cog12 test. An increase in ADAS-Cog12 score is indicative of worsening in a patient's condition. In some embodiments, the reduction or slowing in cognitive decline (or decline in cognitive capacity) in patients being treated with the antibodies of the invention is determined by a Clinical Dementia Rating Scale / Sum of Boxes (CDR-SOB) score. In some embodiments, reduction or slowing in functional decline (or decline in functional ability) in patients being treated with the antibodies of the invention is determined using the Instrumental Activities of Daily Living (iADL) scale. In some embodiments, decline of one or more types is assessed and one or more of the foregoing tests or scales is used to measure reduction or slowing in decline.

**[0012]** An antibody, or antigen-binding fragment thereof, of the invention is administered at a dose that is effective to treat the AD or other amyloidosis, as described herein. Suitable dosages are described herein and can range from about 0.3 mg/kg to 100 mg/kg. In an exemplary embodiment, the dosage is 15 mg/kg. In a further exemplary embodiment, the dosage is 30 mg/kg. In a further exemplary embodiment, the dosage is 45 mg/kg. In some embodiments, the dosage is between 500 mg and 1000mg, for example 500 mg, 700 mg, 720 mg, 750 mg, 800 mg, 820 mg, 900 mg, or between 1000 mg and 2500 mg, for example 1050 mg, 1500 mg, or 2100 mg. In the methods provided herein, a variety of dosage regimens are contemplated including dosage regimens in which the antibody is administered repeatedly, *e.g.*, on a weekly or monthly schedule, over an extended period of time, *e.g.*, months to years.

**[0013]** The humanized monoclonal anti- Abeta antibody of the present disclosure provides a further benefit in that it does not increase the incidence of adverse events such as ARIA-E and ARIA-H. As shown herein, there was no increase in these adverse events in the treatment arm relative to the placebo arm. Thus, the present disclosure further provides methods of treating patients suffering from mild to moderate AD or mild AD without increasing the incidence of adverse events such as ARIA-E and/or ARIA-H.

**[0014]** The present disclosure further provides pharmaceutical formulations suitable for use in the methods of treatment disclosed herein. The pharmaceutical formulations can be formulated for any convenient route of administration, *e.g.*, parenteral or intravenous injection, and will typically include, in addition to the anti-Abeta of the present disclosure, one or more acceptable carriers, excipients, and/or diluents suited to the desired mode of administration. In some embodiments, an antibody of the invention may be formulated for intravenous administration. In some embodiments, an antibody of the invention may be formulated in an arginine buffer, *e.g.*, an arginine succinate buffer. The buffer can contain one or more surfactants, *e.g.*, a polysorbate. In certain embodiments, the buffer concentration is 50 mM or greater. In some embodiments, the pH is between 4.5 and 7.0, e.g., pH 5.5. Further embodiments are described herein. The pharmaceutical formulations can be package in unit dosage forms for ease of use.

**[0015]** Treatment with anti-Abeta antibodies for treatment of AD or other amyloidosis, as described herein, can be combined with other therapy, including one or more anti-Abeta antibodies other than crenezumab. Non-limiting examples of other therapy include neurological drugs, corticosteroids, antibiotics, and antiviral agents. Non-limiting examples of anti-Abeta antibodies other than crenezumab include solanezumab, bapineuzumab, aducanumab, and gantenerumab.

## BRIEF DESCRIPTION OF THE DRAWINGS

**[0016]** **FIG 1** provides the amino acid sequence of Abeta(1-42) (SEQ ID NO:1) with amino acids 13 to 24 underlined.

**[0017]** **FIG 2** provides the amino acid sequence of three heavy chain hypervariable regions (HVR-H1, HVR-H2, and HVR-H3, respectively) and the amino acid sequence of three light chain regions (HVR-L1, HVR-L2, HVR-L3, respectively).

**[0018]** **FIG 3** provides the amino acid sequence of heavy chain (SEQ ID NO:5), comprising the heavy chain variable region spanning amino acids 1 to 112 of SEQ ID NO:5, and light chain (SEQ ID NO:9), comprising the light chain variable region spanning amino acids 1 to 112 of SEQ ID NO:9, of crenezumab. The underlining in SEQ ID NOs:5 and 9 shows the amino acid sequences of the three heavy chain HVR corresponding to SEQ ID NOs:2-4 and the three light chain HVR corresponding to SEQ ID NOs:6-8, respectively.

**[0019]** **FIG 4A-B** provides a summary of the patients enrolled in the clinical trial described in Example 1, tabulating the number of patients enrolled in each arm (treatment

versus placebo), ApoE4 status (ApoE4 negative/ ApoE4 positive), stage of AD (mild or moderate), and MMSE scores at screening, existence and type of concurrent therapy (conmed use) for AD symptoms.

**[0020]** **FIG 5** provides a schematic of the clinical trials described in Example 1, showing the dosing schedule, amount, and route.

**[0021]** **FIG 6A-B** provide data tables showing the change in ADAS-Cog12 scores at 73 weeks relative to baseline, in the treatment arm and the placebo arm. **FIG 6A** provides data for patients with mild to moderate AD, mild AD, moderate AD, and ApoE4 positive and negative patients. **FIG 6B** provides data for patients according to MMSE score.

**[0022]** **FIG 7** provides a chart of the change in ADAS-Cog12 scores for patients with mild AD having an MMSE score between 20 and 26, treated with crenezumab (dark solid line) or placebo (light solid line).

**[0023]** **FIG 8** provides a chart of the change in ADAS-Cog12 scores for patients with mild to moderate AD having an MMSE score between 18 and 26 treated with crenezumab (dark solid line) or placebo (light solid line).

**[0024]** **FIG 9** provides a chart of the change in ADAS-Cog12 scores for ApoE4 positive patients with mild-to-moderate AD treated with crenezumab (dark solid line) or placebo (light solid line).

**[0025]** **FIG 10** provides a chart of the change in ADAS-Cog12 scores across all ApoE4 positive patients and patients with mild AD treated with crenezumab (dark solid line) or placebo (light solid line).

**[0026]** **FIG 11** provides a chart of the change in ADAS-Cog12 scores for patients with mild AD having an MMSE score between 22 and 26, treated with crenezumab or placebo.

**[0027]** **FIG 12A-B** provide data tables showing the change in CDR-SOB scores at 73 weeks relative to baseline, in the treatment arm and the placebo arm. **FIG 12A** provides data for the change in CDR-SOB scores for patients according to MMSE score. **FIG 12B** provides data for CDR-SOB scores as well as CDR Judgment and Problem solving scores and CDR Memory scores for patients with MMSE scores ranging from 18-26, 20-26, and 22-26.

**[0028]** **FIG 13** provides a chart of the change in CDR-SOB scores for patients with mild AD, having an MMSE score of 25 or 26, treated with crenezumab or placebo as indicated.

**[0029]** **FIG 14A-B** provides a summary of the patients enrolled in the clinical trial described in Example 2 at baseline and after treatment, including adverse event data (A) and a

timeline showing when PET scans, MRI scans, and CSF sampling was performed in the clinical trial (B).

**[0030]** **FIG 15A-B** provides charts showing amyloid levels in patients receiving placebo (dashed line) or crenezumab (solid line), as measured by imaging of florbetapir by PET analysis (A) and CSF Abeta levels levels in patients receiving placebo or crenezumab (B).

## DETAILED DESCRIPTION

**[0031]** Unless defined otherwise, technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Singleton *et al.et al.*, Dictionary of Microbiology and Molecular Biology 2nd ed., J. Wiley & Sons (New York, N.Y. 1994), and March, Advanced Organic Chemistry Reactions, Mechanisms and Structure 4th ed., John Wiley & Sons (New York, N.Y. 1992), provide one skilled in the art with a general guide to many of the terms used in the present application.

**Certain Definitions and Abbreviations**

**[0032]** For purposes of interpreting this specification, the following definitions will apply and whenever appropriate, terms used in the singular will also include the plural and vice versa. In the event that any definition set forth below conflicts with any document incorporated herein by reference, the definition set forth below shall control.

**[0033]** As used in this specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "a protein" or an "antibody" includes a plurality of proteins or antibodies, respectively; reference to "a cell" includes mixtures of cells, and the like.

**[0034]** Ranges provided in the specification and appended claims include both end points and all points between the end points. Thus, for example, a range of 2.0 to 3.0 includes 2.0, 3.0, and all points between 2.0 and 3.0.

**[0035]** The phrase "substantially similar," or "substantially the same," as used herein, denotes a sufficiently high degree of similarity between two numeric values (generally one associated with an antibody of the invention and the other associated with a reference/comparator antibody) such that one of skill in the art would consider the difference between the two values to be of little or no biological and/or statistical significance within the context of the biological characteristic measured by said values (*e.g.,* Kd values). The difference between said two values is less than about 50%, less than about 40%, less than

about 30%, less than about 20%, less than about 10% as a function of the value for the reference/comparator antibody.

**[0036]** The term "sample," or "test sample" as used herein, refers to a composition that is obtained or derived from a subject of interest that contains a cellular and/or other molecular entity that is to be characterized and/or identified, for example based on physical, biochemical, chemical and/or physiological characteristics. In one embodiment, the definition encompasses blood and other liquid samples of biological origin and tissue samples such as a biopsy specimen or tissue cultures or cells derived therefrom. The source of the tissue sample may be solid tissue as from a fresh, frozen and/or preserved organ or tissue sample or biopsy or aspirate; blood or any blood constituents; bodily fluids; and cells from any time in gestation or development of the subject or plasma. The term "biological sample" as used herein includes, but is not limited to, blood, serum, plasma, sputum, tissue biopsies (*e.g.*, lung samples), and nasal samples including nasal swabs or nasal polyps.

**[0037]** The term "sample," "biological sample,' or "test sample" includes biological samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as proteins or polynucleotides, or embedding in a semi-solid or solid matrix for sectioning purposes. For the purposes herein a "section" of a tissue sample is meant a single part or piece of a tissue sample, e.g. a thin slice of tissue or cells cut from a tissue sample. Samples include, but are not limited to, whole blood, blood-derived cells, serum, plasma, lymph fluid, synovial fluid, cellular extracts, and combinations thereof. In one embodiment, the sample is a clinical sample. In another embodiment, the sample is used in a diagnostic assay.

**[0038]** In one embodiment, a sample is obtained from a subject or patient prior to treatment with an anti-Abeta antibody. In another embodiment, a sample is obtained from a subject or patient following at least one treatment with an anti-Abeta antibody.

**[0039]** A "reference sample," as used herein, refers to any sample, standard, or level that is used for comparison purposes. In one embodiment, a reference sample is obtained from a healthy and/or non-diseased part of the body (e.g., tissue or cells) of the same subject or patient. In another embodiment, a reference sample is obtained from an untreated tissue and/or cell of the body of the same subject or patient. In yet another embodiment, a reference sample is obtained from a healthy and/or non-diseased part of the body (e.g., tissues or cells) of an individual who is not the subject or patient. In even another embodiment, a reference

sample is obtained from an untreated tissue and/or cell part of the body of an individual who is not the subject or patient.

**[0040]** In certain embodiments, a reference sample is a single sample or combined multiple samples from the same subject or patient that are obtained at one or more different time points than when the test sample is obtained. For example, a reference sample is obtained at an earlier time point from the same subject or patient than when the test sample is obtained. In certain embodiments, a reference sample includes all types of biological samples as defined above under the term "sample" that is obtained from one or more individuals who is not the subject or patient. In certain embodiments, a reference sample is obtained from one or more individuals with amyloidosis, e.g., Alzheimer's Disease, who is not the subject or patient.

**[0041]** In certain embodiments, a reference sample is a combined multiple samples from one or more healthy individuals who are not the subject or patient. In certain embodiments, a reference sample is a combined multiple samples from one or more individuals with a disease or disorder (e.g., amyloidosis such as, for example, Alzheimer's Disease) who are not the subject or patient. In certain embodiments, a reference sample is pooled RNA samples from normal tissues or pooled plasma or serum samples from one or more individuals who are not the subject or patient.

**[0042]** The term "small molecule" refers to an organic molecule having a molecular weight between 50 Daltons to 2500 Daltons.

**[0043]** The terms "antibody" and "immunoglobulin" ("Ig") are used interchangeably in the broadest sense and include, but are not limited to, monoclonal antibodies (for example, full length or intact monoclonal antibodies), polyclonal antibodies, multivalent antibodies, antibodies with polyepitopic specificity, single chain antibodies, multi-specific antibodies (for example, bispecific antibodies, trispecific antibodies, tetraspecific antibodies), and fragments of antibodies, provided they exhibit the desired biological activity. Such antibodies can be chimeric, humanized, human, synthetic, and/or affinity matured. Such antibodies and methods of generating them are described in more detail herein.

**[0044]** "Antibody fragments" comprise only a portion of an intact antibody, wherein the portion preferably retains at least one, and typically most or all, of the functions normally associated with that portion when present in an intact antibody. In one embodiment, an antibody fragment comprises an antigen binding site of the intact antibody and thus retains the ability to bind antigen. In another embodiment, an antibody fragment, for example one

that comprises the Fc region, retains at least one of the biological functions normally associated with the Fc region when present in an intact antibody, such as FcRn binding, antibody half life modulation, ADCC function and complement binding. In one embodiment, an antibody fragment is a monovalent antibody that has an *in vivo* half life substantially similar to an intact antibody. For example, such an antibody fragment may comprise an antigen binding arm linked to an Fc sequence capable of conferring *in vivo* stability to the fragment. Examples of antibody fragments include but are not limited to Fv, Fab, Fab', Fab'-SH, F(ab')2; diabodies; linear antibodies; single-chain antibody molecules (*e.g.* scFv); and multispecific antibodies formed from antibody fragments.

**[0045]** The term "target," as used herein, refers to any native molecule from any vertebrate source, including mammals such as primates (*e.g.* humans) and rodents (*e.g.*, mice and rats), unless otherwise indicated. The term encompasses "full-length," unprocessed target as well as any form of target that results from processing in the cell. The term also encompasses naturally occurring variants of targets, *e.g.*, splice variants or allelic variants.

**[0046]** The terms "amyloid beta," "beta-amyloid," "Abeta," "amyloidβ," and "Aβ", used interchangeably herein, refer to the fragment of amyloid precursor protein ("APP") that is produced upon β-secretase 1 ("BACE1") cleavage of APP, as well as modifications, fragments and any functional equivalents thereof, including, but not limited to, Aβ1-40, and Aβ1-42. Aβ is known to exist in monomeric form, as well as to associate to form oligomers and fibril structures, which may be found as constituent members of amyloid plaque. The structure and sequences of such Aβ peptides are well known to one of ordinary skill in the art and methods of producing said peptides or of extracting them from brain and other tissues are described, for example, in Glenner and Wong, Biochem Biophys Res. Comm. 129: 885-890 (1984). Moreover, Aβ peptides are also commercially available in various forms. An exemplary amino acid sequence of human Aβ1-42 is DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVVIA (SEQ ID NO: 1).

**[0047]** The terms "anti-target antibody" and "an antibody that binds to target" refer to an antibody that is capable of binding the target with sufficient affinity such that the antibody is useful as a diagnostic and/or therapeutic agent in targeting the target. In one embodiment, the extent of binding of an anti-target antibody to an unrelated, non-target protein is less than about 10% of the binding of the antibody to target as measured, *e.g.*, by a radioimmunoassay (RIA) or biacore assay. In certain embodiments, an antibody that binds to a target has a dissociation constant (Kd) of ≤ 1μM, ≤ 100 nM, ≤ 10 nM, ≤ 1 nM, ≤ 0.1 nM, ≤ 0.01 nM, or ≤

0.001 nM (*e.g.*, $10^{-8}$ M or less, *e.g.*, from $10^{-8}$ M to $10^{-13}$ M, *e.g.*, from $10^{-9}$ M to $10^{-13}$ M). In certain embodiments, an anti-target antibody binds to an epitope of a target that is conserved among different species.

**[0048]** "Anti-Abeta immunoglobulin," "anti-Abeta antibody," and "antibody that binds Abeta" are used interchangeably herein, and refer to an antibody that specifically binds to human Abeta. A nonlimiting example of an anti-Abeta antibody is crenezumab. Other non-limiting examples of anti-Abeta antibodies are solanezumab, bapineuzumab, aducanumab, and gantenerumab.

**[0049]** The terms "crenezumab" and "MABT5102A" are used interchangeably herein, and refer to a specific anti-Abeta antibody that binds to monomeric, oligomeric, and fibril forms of Abeta, and which is associated with CAS registry number 1095207. In one embodiment, such antibody comprises HVR region sequences set forth in **FIG 2**. In another such embodiment, such antibody comprises: (1) an HVR-H1 comprising the amino acid sequence SEQ ID NO: 2; (2) an HVR-H2 sequence comprising the amino acid sequence SEQ ID NO: 3; (3) an HVR-H3 sequence comprising the amino acid sequence SEQ ID NO: 4; (4) an HVR-L1 sequence comprising the amino acid sequence SEQ ID NO: 6; (5) an HVR-L2 sequence comprising the amino acid sequence SEQ ID NO: 7; and (6) an HVR-L3 sequence comprising the amino acid sequence SEQ ID NO: 8. In another embodiment, the specific anti-Abeta antibody comprises VH and VL domains having the amino acid sequences set forth in **FIG 3**. In another such embodiment, such specific anti-Abeta antibody comprises a VH domain comprising the amino acid sequence SEQ ID NO: 5 and a VL domain comprising the amino acid sequence SEQ ID NO: 9. In another embodiment, the antibody is an IgG4 antibody. In another such embodiment, the IgG4 antibody comprises a mutation in its constant domain such that serine 228 is instead a proline.

**[0050]** The term "amyloidosis," as used herein, refers to a group of diseases and disorders caused by or associated with amyloid or amyloid-like proteins and includes, but is not limited to, diseases and disorders caused by the presence or activity of amyloid-like proteins in monomeric, fibril, or polymeric state, or any combination of the three, including by amyloid plaques. Such diseases include, but are not limited to, secondary amyloidosis and age-related amyloidosis, such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease ("AD"), diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type),

the Guam Parkinson-Demential complex and other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis, Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), adult onset diabetes, endocrine tumor and senile cardiac amyloidosis, and various eye diseases including macular degeneration, drusen-related optic neuropathy, glaucoma, and cataract due to beta-amyloid deposition.

**[0051]** Glaucoma is a group of diseases of the optic nerve involving loss of retinal ganglion cells (RGCs) in a characteristic pattern of optic neuropathy. RGCs are the nerve cells that transmit visual signals from the eye to the brain. Caspase-3 and Caspase-8, two major enzymes in the apoptotic process, are activated in the process leading to apoptosis of RGCs. Caspase-3 cleaves amyloid precursor protein (APP) to produce neurotoxic fragments, including Abeta. Without the protective effect of APP, Abeta accumulation in the retinal ganglion cell layer results in the death of RGCs and irreversible loss of vision.

**[0052]** Glaucoma is often, but not always, accompanied by an increased eye pressure, which may be a result of blockage of the circulation of aqueous, or its drainage. Although raised intraocular pressure is a significant risk factor for developing glaucoma, no threshold of intraocular pressure can be defined which would be determinative for causing glaucoma. The damage may also be caused by poor blood supply to the vital optic nerve fibers, a weakness in the structure of the nerve, and/or a problem in the health of the nerve fibers themselves. Untreated glaucoma leads to permanent damage of the optic nerve and resultant visual field loss, which can progress to blindness.

**[0053]** The different types of glaucomas are classified as open-angle glaucomas, if the condition is chronic, or closed-angle glaucomas, if acute glaucoma occurs suddenly. Glaucoma usually affects both eyes, but the disease can progress more rapidly in one eye than in the other.

**[0054]** Chronic open-angle glaucoma (COAG), also known as primary open angle glaucoma (POAG), is the most common type of glaucoma. COAG is caused by microscopic blockage in the trabecular meshwork, which decreases the drainage of the aqueous outflow into the Schlemm's canal and raises the intraocular pressure (IOP). POAG usually affects both eyes and is strongly associated with age and a positive family history. Its frequency increases in elderly people as the eye drainage mechanism may gradually become clogged with aging. The increase in intraocular pressure in subjects affected by chronic open-angle glaucoma is not accompanied by any symptoms until the loss is felt on the central visual area.

**[0055]** Acute Angle Closure Glaucoma (AACG) or closed-angle glaucoma is a relatively rare type of glaucoma characterized by a sudden increase in intraocular pressure to 35 to 80 mmHg, leading to severe pain and irreversible loss of vision.. The sudden pressure increase is caused by the closing of the filtering angle and blockage of the drainage channels. Individuals with narrow angles have an increased risk for a sudden closure of the angle. AACG usually occurs monocularly, but the risk exists in both eyes. Age, cataract and pseudoexfoliation are also risk factors since they are associated with enlargement of the lens and crowding or narrowing of the angle. A sudden glaucoma attack may be associated with severe eye pain and headache, inflamed eye, nausea, vomiting, and blurry vision.

**[0056]** Mixed or Combined Mechanism Glaucoma is a mixture or combination of open and closed angle glaucoma. It affects patients with acute ACG whose angle opens after laser iridotomy, but who continue to require medications for IOP control, as well as patients with POAG or pseudoexfoliative glaucoma who gradually develop narrowing of the angle.

**[0057]** Normal tension glaucoma (NTG), also known as low tension glaucoma (LTG), is characterized by progressive optic nerve damage and loss of peripheral vision similar to that seen in other types of glaucoma; however, the intraocular pressure is the normal range or even below normal.

**[0058]** Congenital (infantile) glaucoma is a relatively rare, inherited type of open-angle glaucoma. Insufficient development of the drainage area results in increased pressure in the eye that can lead to the loss of vision from optic nerve damage and to an enlarged eye. Early diagnosis and treatment are critical to preserve vision in infants and children affected by the disease.

**[0059]** Secondary glaucoma may result from an ocular injury, inflammation in the iris of the eye (iritis), diabetes, cataract, or use of steroids in steroid-susceptible individuals. Secondary glaucoma may also be associated with retinal detachment or retinal vein occlusion or blockage.

**[0060]** Pigmentary glaucoma is characterized by the detachment of granules of pigment from the iris. The granules cause blockage of the drainage system of the eye, leading to elevated intraocular pressure and damage to the optic nerve. Exfoliative glaucoma (pseudoexfoliation) is characterized by deposits of flaky material on the anterior capsule and in the angle of the eye. Accumulation of the flaky material blocks the drainage system and raises the eye pressure.

**[0061]** Diagnosis of glaucoma may be made using various tests. Tonometry determines the pressure in the eye by measuring the tone or firmness of its surface. Several types of tonometers are available for this test, the most common being the applanation tonometer. Pachymetry determines the thickness of the cornea which, in turn, measures intraocular pressure. Gonioscopy allows examination of the filtering angle and drainage area of the eye. Gonioscopy can also determine if abnormal blood vessels may be blocking the drainage of the aqueous fluid out of the eye. Ophthalmoscopy allows examination of the optic nerve and can detect nerve fiber layer drop or changes in the optic disc, or indentation (cupping) of this structure, which may be caused by increased intraocular pressure or axonal drop out. Gonioscopy is also useful in assessing damage to the nerve from poor blood flow or increased intraocular pressure. Visual Field testing maps the field of vision, subjectively, which may detect signs of glaucomatous damage to the optic nerve. This is represented by specific patterns of visual field loss. Ocular coherence tomography, an objective measure of nerve fiber layer loss, is carried out by looking at the thickness of the optic nerve fiber layer (altered in glaucoma) via a differential in light transmission through damaged axonal tissue.

**[0062]** An "antibody that binds to the same epitope" as a reference antibody refers to an antibody that blocks binding of the reference antibody to its antigen in a competition assay by 50% or more, and conversely, the reference antibody blocks binding of the antibody to its antigen in a competition assay by 50% or more. An exemplary competition assay is provided herein.

**[0063]** The term "therapeutic agent" refers to any agent that is used to treat a disease, including but not limited to an agent that treats a symptom of the disease.

**[0064]** As used herein, "treatment" (and grammatical variations thereof such as "treat" or "treating") refers to clinical intervention in an attempt to alter the natural course of the individual being treated, and can be performed during the course of clinical pathology. Desirable effects of treatment include, but are not limited to, alleviation or amelioration of one or more symptoms, diminishment of or delay in the appearance of or worsening of any direct or indirect pathological consequences of the disease, decrease of the rate of disease progression, and amelioration or palliation of the disease state. In some embodiments, antibodies are used to delay development of a disease or to slow the progression of a disease.

**[0065]** The term "treatment emergent" as used herein refers to an event that occurs after a first dose of a therapeutic agent is administered. For example, a "treatment emergent adverse

event" is an event that is identified upon or after the first dose of a treatment in a clinical study.

**[0066]** "Treatment regimen" refers to a combination of dosage, frequency of administration, or duration of treatment, with or without addition of a second medication.

**[0067]** "Effective treatment regimen" refers to a treatment regimen that will offer beneficial response to a patient receiving the treatment.

**[0068]** "Modifying a treatment" refers to changing the treatment regimen including, changing dosage, frequency of administration, or duration of treatment, and/or addition of a second medication.

**[0069]** An "effective amount" or "effective dose" of an agent refers to an amount or dose effective, for periods of time necessary, to achieve the desired result. For example, a "therapeutically effective amount" is an amount effective, for periods of time necessary, to treat the indicated disease, condition, clinical pathology, or symptom, *i.e.*, to modify the course of progression of AD and/or to alleviate and/or prevent one or more symptoms of AD.

**[0070]** "Affinity" or "binding affinity" refers to the strength of the sum total of noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen). Unless indicated otherwise, as used herein, "binding affinity" refers to intrinsic binding affinity which reflects a 1:1 interaction between members of a binding pair (*e.g.*, antibody and antigen binding arm). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (Kd). Affinity can be measured by common methods known in the art, including those described herein, any of which can be used for purposes of the present invention. Specific illustrative and exemplary embodiments for measuring binding affinity are described herein.

**[0071]** An "affinity matured" antibody refers to an antibody with one or more alterations in one or more hypervariable regions (HVRs), compared to a parent antibody which does not possess such alterations, such alterations resulting in an improvement in the affinity of the antibody for antigen.

**[0072]** As used herein, the term "patient" refers to any single subject for which treatment is desired. In certain embodiments, the patient herein is a human.

**[0073]** A "subject" herein is typically a human. In certain embodiments, a subject is a non-human mammal. Exemplary non-human mammals include laboratory, domestic, pet, sport, and stock animals, *e.g.*, mice, cats, dogs, horses, and cows. Typically, the subject is eligible for treatment, *e.g.*, displays one or more indicia of disease. Generally, such subject or

patient is eligible for treatment for amyloidosis, e.g., AD. In one embodiment, such eligible subject or patient is one that is experiencing or has experienced one or more signs, symptoms, or other indicators of AD or has been diagnosed with AD, whether, for example, newly diagnosed, previously diagnosed or at risk for developing AD. Diagnosis of AD may be made based on clinical history, clinical examination, and established imaging modalities. A "patient" or "subject" herein includes any single human subject eligible for treatment who is experiencing or has experienced one or more signs, symptoms, or other indicators of AD. Intended to be included as a subject are any subjects involved in clinical research trials, or subjects involved in epidemiological studies, or subjects once used as controls. The subject may have been previously treated with an anti-Abeta antibody, or antigen-binding fragment thereof, or another drug, or not so treated. The subject may be naïve to an additional drug(s) being used when the treatment herein is started, i.e., the subject may not have been previously treated with, for example, a therapy other than anti-Abeta at "baseline" (i.e., at a set point in time before the administration of a first dose of anti-Abeta in the treatment method herein, such as the day of screening the subject before treatment is commenced). Such "naïve" subjects are generally considered to be candidates for treatment with such additional drug(s).

**[0074]** As used herein, "lifetime" of a subject refers to the remainder of the life of the subject after starting treatment.

**[0075]** The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.,* the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigen. Furthermore, in contrast to polyclonal antibody preparations that typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen.

**[0076]** The monoclonal antibodies herein specifically include "chimeric" antibodies in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; and Morrison *et al.,* Proc. Natl. Acad. Sci. USA 81:6851-6855 (1984)).

**[0077]** The "class" of an antibody refers to the type of constant domain or constant region possessed by its heavy chain. There are five major classes of antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (or "isotypes"), *e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The heavy chain constant domains that correspond to the different classes of immunoglobulins are called α, δ, ε, γ, and μ, respectively.

**[0078]** "Humanized" forms of non-human (*e.g.,* murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some instances, framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FRs are those of a human immunoglobulin lo sequence. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.,* Nature 321:522-525 (1986); Riechmann *et al.,* Nature 332:323-329 (1988); and Presta, Curr. Op. Struct. Biol. 2:593-596 (1992). See also the following review articles and references cited therein: Vaswani and Hamilton, Ann. Allergy, Asthma & Immunol. 1: 105-115 (1998); Harris, Biochem. Soc. Transactions 23:1035-1038 (1995); Hurle and Gross, Curr. Op. Biotech. 5:428-433 (1994).

**[0079]** A "human antibody" is one which comprises an amino acid sequence corresponding to that of an antibody produced by a human or a human cell and/or has been derived from a non-human source that utilizes human antibody repertoires or other human antibody-encoding sequences, for example made using any of the techniques for making human antibodies as disclosed herein. Such techniques include, but are not limited to, screening human-derived combinatorial libraries, such as phage display libraries (see, *e.g.,* Marks *et al.*, *J. Mol. Biol.,* 222: 581-597 (1991) and Hoogenboom *et al.*, *Nucl. Acids Res.*, 19: 4133-4137 (1991)); using human myeloma and mouse-human heteromyeloma cell lines for

the production of human monoclonal antibodies (see, *e.g.,* Kozbor *J. Immunol.*, 133: 3001 (1984); Brodeur *et al.*, *Monoclonal Antibody Production Techniques and Applications*, pp. 55-93 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al.*, *J. Immunol.*, 147: 86 (1991)); and generating monoclonal antibodies in transgenic animals (*e.g.,* mice) that are capable of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production (see, *e.g.,* Jakobovits *et al.*, *Proc. Natl. Acad. Sci USA,* 90: 2551 (1993); Jakobovits *et al.*, *Nature,* 362: 255 (1993); Bruggermann *et al.*,*Year in Immunol.,* 7: 33 (1993)). This definition of a human antibody specifically excludes a humanized antibody comprising antigen-binding residues from a non-human animal.

**[0080]** An "isolated" antibody is one which has been identified and separated and/or recovered from a component of its natural environment. Contaminant components of its natural environment are materials which would interfere with diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or nonproteinaceous solutes. In some embodiments, an antibody is purified to greater than 95% or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC). For review of methods for assessment of antibody purity, see, *e.g.*, Flatman *et al.*, J. Chromatogr. B 848:79-87 (2007).

**[0081]** The term "variable region" or "variable domain" refers to the domain of an antibody heavy or light chain that is involved in binding the antibody to antigen. The variable domains of the heavy chain and light chain (VH and VL, respectively) of a native antibody generally have similar structures, with each domain comprising four conserved framework regions (FRs) and three hypervariable regions (HVRs). (See, *e.g.*, Kindt *et al.* Kuby Immunology, 6th ed., W.H. Freeman and Co., page 91 (2007).) A single VH or VL domain may be sufficient to confer antigen-binding specificity. Furthermore, antibodies that bind a particular antigen may be isolated using a VH or VL domain from an antibody that binds the antigen to screen a library of complementary VL or VH domains, respectively. *See, e.g.*, Portolano *et al.*, J. Immunol. 150:880-887 (1993); Clarkson *et al.*, Nature 352:624-628 (1991).

**[0082]** The term "hypervariable region," "HVR," or "HV," when used herein refers to the regions of an antibody variable domain which are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six hypervariable regions; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). A number of hypervariable region

delineations are in use and are encompassed herein. The Kabat Complementarity Determining Regions (CDRs) are based on sequence variability and are the most commonly used (Kabat *et al.,* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md. (1991)). Chothia refers instead to the location of the structural loops (Chothia and Lesk J. Mol. Biol. 196:901-917 (1987)). The AbM hypervariable regions represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody modeling software. The "contact" hypervariable regions are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

| Loop | Kabat | AbM | Chothia | Contact | |
|---|---|---|---|---|---|
| L1 | L24-L34 | L24-L34 | L26-L32 | L30-L36 | |
| L2 | L50-L56 | L50-L56 | L50-L52 | L46-L55 | |
| L3 | L89-L97 | L89-L97 | L91-L96 | L89-L96 | |
| H1 | H31-H35B | H26-H35B | H26-H32 | H30-H35B | (Kabat Numbering) |
| H1 | H31-H35 | H26-H35 | H26-H32 | H30-H35 | (Chothia Numbering) |
| H2 | H50-H65 | H50-H58 | H53-H55 | H47-H58 | |
| H3 | H95-H102 | H95-H102 | H96-H101 | H93-H101 | |

**[0083]** Hypervariable regions may comprise "extended hypervariable regions" as follows: 24-36 or 24-34 (L1), 46-56 or 49-56 or 50-56 or 52-56 (L2) and 89-97 (L3) in the VL and 26-35 (H1), 50-65 or 49-65 (H2) and 93-102, 94-102 or 95-102 (H3) in the VH. The variable domain residues are numbered according to Kabat *et al., supra* for each of these definitions.

**[0084]** "Framework" or "FR" residues are those variable domain residues other than the hypervariable region residues as herein defined. The FR of a variable domain generally consists of four FR domains: FR1, FR2, FR3, and FR4. Accordingly, the HVR and FR sequences generally appear in the following sequence in VH (or VL): FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4.

**[0085]** An "acceptor human framework" for the purposes herein is a framework comprising the amino acid sequence of a light chain variable domain (VL) framework or a heavy chain variable domain (VH) framework derived from a human immunoglobulin framework or a human consensus framework, as defined below. An acceptor human framework "derived from" a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain amino acid sequence changes. In some embodiments, the number of amino acid changes are 10 or less, 9

or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. In some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

**[0086]** A "human consensus framework" is a framework which represents the most commonly occurring amino acid residue in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat *et al.* Sequences of Proteins of Immunological Interest, Fifth Edition, NIH Publication 91-3242, Bethesda MD (1991), vols. 1-3. *et al.et al.*

**[0087]** The term "Amyloid-Related Imaging Abnormality – Edema" or "ARIA-E" encompasses cerebral vasogenic edema and sulcal effusion.

**[0088]** The term "Amyloid-Related Imaging Abnormality – Hemorrhage" or "ARIA-H" encompasses microhemorrhage and superficial siderosis of the central nervous system.

**[0089]** "Apolipoprotein E4 carrier" or "ApoE4 carrier," used interchangeably herein with "apolipoprotein E4 positive" or "ApoE4 positive," refers to an individual having at least one apolipoprotein E4 (or "ApoE4") allele. An individual with zero ApoE4 alleles is referred to herein as being "ApoE4 negative" or an "ApoE4 non-carrier." *See also* Prekumar, *et al.*, 1996, Am. J Pathol. 148:2083−95.

**[0090]** The term "cerebral vasogenic edema" refers to an excess accumulation of intravascular fluid or protein in the intracellular or extracellular spaces of the brain. Cerebral vasogenic edema is detectable by, *e.g.*, brain MRI, including, but not limited to FLAIR MRI, and can be asymptomatic ("asymptomatic vasogenic edema") or associated with neurological symptoms, such as confusion, dizziness, vomiting, and lethargy ("symptomatic vasogenic edema") (*see* Sperling *et al.* Alzheimer's & Dementia, 7:367, 2011).

**[0091]** The term "cerebral macrohemorrhage" refers to an intracranial hemorrhage, or bleeding in the brain, of an area that is more than about 1 cm in diameter. Cerebral macrohemorrhage is detectable by, *e.g.*, brain MRI, including but not limited to T2*-weighted GRE MRI, and can be asymptomatic ("asymptomatic macrohemorrhage") or associated with symptoms such as transient or permanent focal motor or sensory impairment, ataxia, aphasia, and dysarthria ("symptomatic macrohemorrhage") (see, *e.g.*, Chalela JA, Gomes J. Expert Rev. Neurother. 2004 4:267, 2004 and Sperling *et al.* Alzheimer's & Dementia, 7:367, 2011).

**[0092]** The term "cerebral microhemorrhage" refers to an intracranial hemorrhage, or bleeding in the brain, of an area that is less than about 1 cm in diameter. Cerebral

microhemorrhage is detectable by, *e.g.*, brain MRI, including, but not limited to T2*-weighted GRE MRI, and can be asymptomatic ("asymptomatic microhemorrhage") or can potentially be associated with symptoms such as transient or permanent focal motor or sensory impairment, ataxia, aphasia, and dysarthria ("symptomatic microhemorrhage"). *See, e.g.*, Greenberg, *et al.*, 2009, Lancet Neurol. 8:165−74.

**[0093]** The term "sulcal effusion" refers to an effusion of fluid in the furrows, or sulci, of the brain. Sulcal effusions are detectable by, *e.g.*, brain MRI, including but not limited to FLAIR MRI. *See* Sperling *et al.* Alzheimer's & Dementia, 7:367, 2011.

**[0094]** The term "superficial siderosis of the central nervous system" refers to bleeding or hemorrhage into the subarachnoid space of the brain and is detectable by, *e.g.*, brain MRI, including but not limited to T2*-weighted GRE MRI. Symptoms indicative of superficial siderosis of the central nervous system include sensorineural deafness, cerebellar ataxia, and pyramidal signs. *See* Kumara-N, Am J Neuroradiol. 31:5, 2010.

**[0095]** The term "progression" as used herein refers to the worsening of a disease over time. The "progression rate" or "rate of progression" of a disease refers to how fast or slow a disease develops over time in a patient diagnosed with the disease. The progression rate of a disease can be represented by measurable changes over time of particular characteristics of the disease. A patient carrying particular genetic trait is said to have, or more likely to have, "increased progression rate" if her disease state progresses faster than those patients without such genetic trait. On the other hand, a patient responding to a therapy is said to have, or more likely to have, "decreased progression rate" if her disease progression slows down after the therapy, when compared to her disease state prior to the treatment or to other patients without the treatment.

**[0096]** "More likely to respond" as used herein refers to patients that are most likely to demonstrate a slowing down or prevention of progression of amyloidosis, e.g., AD. With regard to AD, "more likely to respond" refers to patients that are most likely to demonstrate a reduction in loss of function or cognition with treatment. The phrase "responsive to" in the context of the present invention indicates that a patient suffering from, being suspected to suffer or being prone to suffer from, or diagnosed with a disorder as described herein, shows a response to anti-Abeta treatment.

**[0097]** The phrase "selecting a patient" or "identifying a patient" as used herein refers to using the information or data generated relating to the presence of an allele in a sample of a patient to identify or select the patient as more likely to benefit to benefit from a treatment

comprising anti-Abeta antibody. The information or data used or generated may be in any form, written, oral or electronic. In some embodiments, using the information or data generated includes communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof. In some embodiments, communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof are performed by a computing device, analyzer unit or combination thereof. In some further embodiments, communicating, presenting, reporting, storing, sending, transferring, supplying, transmitting, dispensing, or combinations thereof are performed by a laboratory or medical professional. In some embodiments, the information or data includes an indication that a specific allele is present or absent in the sample. In some embodiments, the information or data includes an indication that the patient is more likely to respond to a therapy comprising anti-Abeta.

**[0098]** "Effector functions" refer to those biological activities attributable to the Fc region of an antibody, which vary with the antibody isotype. Examples of antibody effector functions include: C1q binding and complement dependent cytotoxicity (CDC); Fc receptor binding; antibody-dependent cell-mediated cytotoxicity (ADCC); phagocytosis; down regulation of cell surface receptors (*e.g.* B cell receptor); and B cell activation. It is known in the art that wild-type IgG4 antibodies have less effector function than wild-type IgG1 antibodies.

**[0099]** The term "Fc region" herein is used to define a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD, 1991.

**[0131]** The terms "full length antibody," "intact antibody," and "whole antibody" are used herein interchangeably to refer to an antibody having a structure substantially similar to a native antibody structure or having heavy chains that contain an Fc region as defined herein.

**[0132]** The terms "host cell," "host cell line," and "host cell culture" are used interchangeably and refer to cells into which exogenous nucleic acid has been introduced,

including the progeny of such cells. Host cells include "transformants" and "transformed cells," which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell, but may contain mutations. Mutant progeny that have the same function or biological activity as screened or selected for in the originally transformed cell are included herein.

**[0133]** An "immunoconjugate" is an antibody conjugated to one or more heterologous molecule(s), including but not limited to a further therapeutic agent.

**[0134]** An "isolated" nucleic acid refers to a nucleic acid molecule that has been separated from a component of its natural environment. An isolated nucleic acid includes a nucleic acid molecule contained in cells that ordinarily contain the nucleic acid molecule, but the nucleic acid molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location.

**[0135]** "Isolated nucleic acid encoding an anti-Abeta antibody" refers to one or more nucleic acid molecules encoding antibody heavy and light chains (or fragments thereof), including such nucleic acid molecule(s) in a single vector or separate vectors, and such nucleic acid molecule(s) present at one or more locations in a host cell.

**[0136]** The term "early Alzheimer's Disease" or "early AD" as used herein (e.g., a "patient diagnosed with early AD" or a "patient suffering from early AD") includes patients with mild cognitive impairement, such as a memory deficit, due to AD and patients having AD biomarkers, for example amyloid positive patients.

**[0137]** The term "mild Alzheimer's Disease" or "mild AD" as used herein (*e.g.*, a "patient diagnosed with mild AD") refers to a stage of AD characterized by an MMSE score of 20 to 26.

**[0138]** The term "mild to moderate Alzheimer's Disease" or "mild to moderate AD" as used herein encompasses both mild and moderate AD, and is characterized by an MMSE score of 18 to 26.

**[0139]** The term "moderate Alzheimer's Disease" or "moderate AD" as used herein (*e.g.*, a "patient diagnosed with moderate AD") refers to a stage of AD characterized by an MMSE score of 18 to 19.

**[0140]** A "naked antibody" refers to an antibody that is not conjugated to a heterologous moiety (*e.g.*, a further therapeuticmoiety) or radiolabel. The naked antibody may be present in a pharmaceutical formulation.

**[0141]** "Native antibodies" refer to naturally occurring immunoglobulin molecules with varying structures. For example, native IgG antibodies are heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light chains and two identical heavy chains that are disulfide-bonded. From N- to C-terminus, each heavy chain has a variable region (VH), also called a variable heavy domain or a heavy chain variable domain, followed by three constant domains (CH1, CH2, and CH3). Similarly, from N- to C-terminus, each light chain has a variable region (VL), also called a variable light domain or a light chain variable domain, followed by a constant light (CL) domain. The light chain of an antibody may be assigned to one of two types, called kappa (κ) and lambda (λ), based on the amino acid sequence of its constant domain.

**[0142]** The term "package insert" is used to refer to instructions customarily included in commercial packages of therapeutic products, that contain information about the indications, usage, dosage, administration, combination therapy, contraindications and/or warnings concerning the use of such therapeutic products. The term "package insert" is also used to refer to instructions customarily included in commercial packages of diagnostic products that contain information about the intended use, test principle, preparation and handling of reagents, specimen collection and preparation, calibration of the assay and the assay procedure, performance and precision data such as sensitivity and specificity of the assay.

**[0143]** "Percent (%) amino acid sequence identity" with respect to a reference polypeptide sequence is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the reference polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For purposes herein, however, % amino acid sequence identity values are generated using the sequence comparison computer program ALIGN-2. The ALIGN-2 sequence comparison computer program was authored by Genentech, Inc., and the source code has been filed with user documentation in the U.S. Copyright Office, Washington D.C., 20559, where it is registered

under U.S. Copyright Registration No. TXU510087. The ALIGN-2 program is publicly available from Genentech, Inc., South San Francisco, California, or may be compiled from the source code. The ALIGN-2 program should be compiled for use on a UNIX operating system, including digital UNIX V4.0D. All sequence comparison parameters are set by the ALIGN-2 program and do not vary.

**[0144]** In situations where ALIGN-2 is employed for amino acid sequence comparisons, the % amino acid sequence identity of a given amino acid sequence A to, with, or against a given amino acid sequence B (which can alternatively be phrased as a given amino acid sequence A that has or comprises a certain % amino acid sequence identity to, with, or against a given amino acid sequence B) is calculated as follows:

100 times the fraction (X/Y)

where X is the number of amino acid residues scored as identical matches by the sequence alignment program ALIGN-2 in that program's alignment of A and B, and where Y is the total number of amino acid residues in B. It will be appreciated that where the length of amino acid sequence A is not equal to the length of amino acid sequence B, the % amino acid sequence identity of A to B will not equal the % amino acid sequence identity of B to A. Unless specifically stated otherwise, all % amino acid sequence identity values used herein are obtained as described in the immediately preceding paragraph using the ALIGN-2 computer program.

**[0145]** The terms "pharmaceutical formulation" and "pharmaceutical composition" are used interchangeably herein and refer to a preparation which is in such form as to permit the biological activity of an active ingredient contained therein to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered.

**[0146]** A "pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical formulation, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

**[0147]** The term "vector," as used herein, refers to a nucleic acid molecule capable of propagating another nucleic acid to which it is linked. The term includes the vector as a self-replicating nucleic acid structure as well as the vector incorporated into the genome of a host cell into which it has been introduced. Certain vectors are capable of directing the expression

of nucleic acids to which they are operatively linked. Such vectors are referred to herein as "expression vectors."

**[0148]** An "imaging agent" is a compound that has one or more properties that permit its presence and/or location to be detected directly or indirectly. Examples of such imaging agents include proteins and small molecule compounds incorporating a labeled moiety that permits detection.

**[0149]** A "label" is a marker coupled with a molecule to be used for detection or imaging. Examples of such labels include: a radiolabel, a fluorophore, a chromophore, or an affinity tag. In one embodiment, the label is a radiolabel used for medical imaging, for example tc99m or I123, or a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123 again, iodine-131, indium-111, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese, iron, etc.

**METHODS AND COMPOSITIONS**

**[0150]** The present disclosure provides compositions and methods for the treatment, prognosis, selection and/or identification of patients at risk for or having amyloidosis. In one aspect, the invention is based, in part, on improved methods of treatment.

**[0151]** In certain embodiments, antibodies that bind to Abeta are provided. Antibodies of the invention are useful, *e.g.*, for the diagnosis or treatment of Alzheimer's Disease ("AD") and other diseases.

**Exemplary Antibodies**

**[0152]** In one aspect, the invention provides isolated antibodies that bind to Abeta. In certain embodiments, the invention provides an anti-Abeta antibody that can bind to monomeric, oligomeric and fibril forms of human Abeta with good affinity. In one embodiment, the anti-Abeta antibody is an antibody that binds to an epitope of Abeta within residues 13-24 of Abeta. In one such embodiment, the antibody is crenezumab.

**[0153]** In one embodiment, the antibody comprises the heavy chain amino acid sequence set forth in SEQ ID NO:5 and the light chain amino acid sequence set forth in SEQ ID NO:9. In another embodiment, the antibody comprises the heavy chain variable region of amino acids 1 to 112 of the amino acid sequence set forth in SEQ ID NO:5 and the light chain variable region of amino acids 1 to 112 of the amino acid sequence set forth in SEQ ID NO:9. In another embodiment, the antibody comprises the HVR sequences of SEQ ID NO:5 and SEQ ID NO:9. In another embodiment, the antibody comprises HVR sequences that are

95%, 96%, 97%, 98%, or 99% or more identical to the HVR sequences of SEQ ID NO:5 and SEQ ID NO:9.

**[0154]** In any of the above embodiments, an anti-Abeta antibody is humanized. In one embodiment, an anti-Abeta antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, *e.g.* a human immunoglobulin framework or a human consensus framework.

**[0155]** In another aspect, an anti-Abeta antibody comprises a heavy chain variable domain (VH) sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to amino acids 1 to 112 of the amino acid sequence of SEQ ID NO:5. In certain embodiments, a VH sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Abeta antibody comprising that sequence retains the ability to bind to Abeta. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:5. In certain embodiments, substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-Abeta antibody comprises the VH sequence in SEQ ID NO:5, including post-translational modifications of that sequence.

**[0156]** In another aspect, an anti-Abeta antibody is provided, wherein the antibody comprises a light chain variable domain (VL) having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to amino acids 1 to 112 of the amino acid sequence of SEQ ID NO:9. In certain embodiments, a VL sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% identity contains substitutions (*e.g.*, conservative substitutions), insertions, or deletions relative to the reference sequence, but an anti-Abeta antibody comprising that sequence retains the ability to bind to Abeta. In certain embodiments, a total of 1 to 10 amino acids have been substituted, inserted and/or deleted in SEQ ID NO:9. In certain embodiments, the substitutions, insertions, or deletions occur in regions outside the HVRs (*i.e.*, in the FRs). Optionally, the anti-Abeta antibody comprises the VL sequence in SEQ ID NO:9, including post-translational modifications of that sequence.

**[0157]** In another aspect, an anti-Abeta antibody is provided, wherein the antibody comprises a VH as in any of the embodiments provided above, and a VL as in any of the embodiments provided above.

**[0158]** In a further aspect, the invention provides an antibody that binds to the same epitope as an anti-Abeta antibody provided herein. For example, in certain embodiments, an antibody is provided that binds to the same epitope as an anti-Abeta antibody comprising a VH sequence in SEQ ID NO:5 and a VL sequence in SEQ ID NO:9.

**[0159]** In a further aspect of the invention, an anti-Abeta antibody according to any of the above embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. In one embodiment, an anti-Abeta antibody is an antibody fragment, *e.g.*, a Fv, Fab, Fab', scFv, diabody, or F(ab')2 fragment. In another embodiment, the antibody is a full length antibody, *e.g.*, an intact IgG4 antibody or other antibody class or isotype as defined herein. In another embodiment, the antibody is a bispecific antibody.

**[0160]** In a further aspect, an anti-Abeta antibody according to any of the above embodiments may incorporate any of the features, singly or in combination, as described in Sections 1-7 below.

**[0161]** In one embodiment, the anti-Abeta antibody comprises a HVR-L1 comprising amino acid sequence SEQ ID NO:6; an HVR-L2 comprising amino acid sequence SEQ ID NO:7; an HVR-L3 comprising amino acid sequence SEQ ID NO: 8; an HVR-H1 comprising amino acid sequence SEQ ID NO:2; an HVR-H2 comprising amino acid sequence SEQ ID NO: 3; and an HVR-H3 comprising amino acid sequence SEQ ID NO: 4.

**[0162]** In another embodiment, the antibody comprises the heavy and light sequences SEQ ID NO:5 and SEQ ID NO:9.

**[0163]** In another embodiment, the antibody comprises the variable region sequences in SEQ ID NO:5 and SEQ ID NO:9.

**[0164]** In any of the above embodiments, an anti-Abeta antibody can be humanized. In one embodiment, an anti-Abeta antibody comprises HVRs as in any of the above embodiments, and further comprises an acceptor human framework, *e.g.* a human immunoglobulin framework or a human consensus framework.

***1. Antibody Affinity***

**[0165]** In certain embodiments, an antibody provided herein has a dissociation constant (Kd) of $\leq 1\mu M$, $\leq 100$ nM, $\leq 10$ nM, $\leq 1$ nM, $\leq 0.1$ nM, $\leq 0.01$ nM, or $\leq 0.001$ nM (*e.g.* $10^{-8}$ M or less, *e.g.* from $10^{-8}$ M to $10^{-13}$ M, *e.g.*, from $10^{-9}$ M to $10^{-13}$ M).

**[0166]** In one embodiment, Kd is measured by a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and its antigen as described by the following assay. Solution binding affinity of Fabs for antigen is measured by equilibrating

Fab with a minimal concentration of ($^{125}$I)-labeled antigen in the presence of a titration series of unlabeled antigen, then capturing bound antigen with an anti-Fab antibody-coated plate (*see, e.g.*, Chen *et al.et al.*, J. Mol. Biol. 293:865-881(1999)). To establish conditions for the assay, MICROTITER® multi-well plates (Thermo Scientific) are coated overnight with 5 μg/ml of a capturing anti-Fab antibody (Cappel Labs) in 50 mM sodium carbonate (pH 9.6), and subsequently blocked with 2% (w/v) bovine serum albumin in PBS for two to five hours at room temperature (approximately 23°C). In a non-adsorbent plate (Nunc #269620), 100 pM or 26 pM [125I]-antigen are mixed with serial dilutions of a Fab of interest (*e.g.*, consistent with assessment of the anti-VEGF antibody, Fab-12, in Presta *et al.*, Cancer Res. 57:4593-4599 (1997)). The Fab of interest is then incubated overnight; however, the incubation may continue for a longer period (*e.g.*, about 65 hours) to ensure that equilibrium is reached. Thereafter, the mixtures are transferred to the capture plate for incubation at room temperature (*e.g.*, for one hour). The solution is then removed and the plate washed eight times with 0.1% polysorbate 20 (TWEEN-20®) in PBS. When the plates have dried, 150 μl/well of scintillant (MICROSCINT-20 TM; Packard) is added, and the plates are counted on a TOPCOUNT TM gamma counter (Packard) for ten minutes. Concentrations of each Fab that give less than or equal to 20% of maximal binding are chosen for use in competitive binding assays.

**[0167]** According to another embodiment, Kd is measured using surface plasmon resonance assays using a BIACORE®-2000 or a BIACORE ®-3000 (BIAcore, Inc., Piscataway, NJ) at 25°C with immobilized antigen CM5 chips at ~10 response units (RU). Briefly, carboxymethylated dextran biosensor chips (CM5, BIACORE, Inc.) are activated with N-ethyl-N'- (3-dimethylaminopropyl)-carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) according to the supplier's instructions. Antigen is diluted with 10 mM sodium acetate, pH 4.8, to 5 μg/ml (~0.2 μM) before injection at a flow rate of 5 μl/minute to achieve approximately 10 response units (RU) of coupled protein. Following the injection of antigen, 1 M ethanolamine is injected to block unreacted groups. For kinetics measurements, two-fold serial dilutions of Fab (0.78 nM to 500 nM) are injected in PBS with 0.05% polysorbate 20 (TWEEN-20TM) surfactant (PBST) at 25°C at a flow rate of approximately 25 μl/min. Association rates (kon) and dissociation rates (koff) are calculated using a simple one-to-one Langmuir binding model (BIACORE ® Evaluation Software version 3.2) by simultaneously fitting the association and dissociation sensorgrams. The equilibrium dissociation constant (Kd) is calculated as the ratio koff/kon. See, *e.g.*, Chen *et*

*al.*, J. Mol. Biol. 293:865-881 (1999). If the on-rate exceeds $10^6$ $M^{-1}$ $s^{-1}$ by the surface plasmon resonance assay above, then the on-rate can be determined by using a fluorescent quenching technique that measures the increase or decrease in fluorescence emission intensity (excitation = 295 nm; emission = 340 nm, 16 nm band-pass) at 25°C of a 20 nM antigen antibody (Fab form) in PBS, pH 7.2, in the presence of increasing concentrations of antigen as measured in a spectrometer, such as a stop-flow equipped spectrophometer (Aviv Instruments) or a 8000-series SLM-AMINCO TM spectrophotometer (ThermoSpectronic) with a stirred cuvette.

***2. Antibody Fragments***

**[0168]** In certain embodiments, an antibody provided herein is an antibody fragment. Antibody fragments include, but are not limited to, Fab, Fab', Fab'-SH, F(ab')2, Fv, and scFv fragments, and other fragments described below. For a review of certain antibody fragments, see Hudson *et al.* Nat. Med. 9:129-134 (2003). For a review of scFv fragments, see, *e.g.*, Pluckthün, in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., (Springer-Verlag, New York), pp. 269-315 (1994); see also WO 93/16185; and U.S. Patent Nos. 5,571,894 and 5,587,458. For discussion of Fab and F(ab')2 fragments comprising salvage receptor binding epitope residues and having increased in vivo half-life, see U.S. Patent No. 5,869,046.

**[0169]** Diabodies are antibody fragments with two antigen-binding sites that may be bivalent or bispecific. See, for example, EP 404,097; WO 1993/01161; Hudson *et al.*, Nat. Med. 9:129-134 (2003); and Hollinger *et al.*, Proc. Natl. Acad. Sci. USA 90: 6444-6448 (1993). Triabodies and tetrabodies are also described in Hudson *et al.*, Nat. Med. 9:129-134 (2003).

**[0170]** Single-domain antibodies are antibody fragments comprising all or a portion of the heavy chain variable domain or all or a portion of the light chain variable domain of an antibody. In certain embodiments, a single-domain antibody is a human single-domain antibody (Domantis, Inc., Waltham, MA; see, *e.g.*, U.S. Patent No. 6,248,516 B1). In certain embodiments, two or more single-domain antibodies may be joined together to form an immunoglobulin construct with multivalent affinity (*i.e.*, the N- or C-terminus of a first single-domain antibody may be fused or otherwise joined to the N- or C-terminus of a second single-domain antibody).

**[0171]** Antibody fragments can be made by various techniques, including but not limited to proteolytic digestion of an intact antibody as well as production by recombinant host cells (*e.g. E. coli* or phage), as described herein.

***3. Chimeric and Humanized Antibodies***

**[0172]** In certain embodiments, an antibody provided herein is a chimeric antibody. Certain chimeric antibodies are described, *e.g.*, in U.S. Patent No. 4,816,567; and Morrison *et al.*, Proc. Natl. Acad. Sci. USA, 81:6851-6855 (1984)). In one example, a chimeric antibody comprises a non-human variable region (*e.g.*, a variable region derived from a mouse, rat, hamster, rabbit, or non-human primate, such as a monkey) and a human constant region. In a further example, a chimeric antibody is a "class switched" antibody in which the class or subclass has been changed from that of the parent antibody. Chimeric antibodies include antigen-binding fragments thereof.

**[0173]** In certain embodiments, a chimeric antibody is a humanized antibody. Typically, a non-human antibody is humanized to reduce immunogenicity to humans, while retaining the specificity and affinity of the parental non-human antibody. Generally, a humanized antibody comprises one or more variable domains in which HVRs, *e.g.*, CDRs, (or portions thereof) are derived from a non-human antibody, and FRs (or portions thereof) are derived from human antibody sequences. A humanized antibody optionally will also comprise at least a portion of a human constant region. In some embodiments, some FR residues in a humanized antibody are substituted with corresponding residues from a non-human antibody (*e.g.*, the antibody from which the HVR residues are derived), *e.g.*, to restore or improve antibody specificity or affinity.

**[0174]** Humanized antibodies and methods of making them are reviewed, *e.g.*, in Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008), and are further described, *e.g.*, in Riechmann *et al.*, Nature 332:323-329 (1988); Queen *et al.*, Proc. Nat'l Acad. Sci. USA 86:10029-10033 (1989); US Patent Nos. 5, 821,337, 7,527,791, 6,982,321, and 7,087,409; Kashmiri *et al.*, Methods 36:25-34 (2005) (describing SDR (a-CDR) grafting); Padlan, Mol. Immunol. 28:489-498 (1991) (describing "resurfacing"); Dall'Acqua *et al.*, Methods 36:43-60 (2005) (describing "FR shuffling"); and Osbourn *et al.*, Methods 36:61-68 (2005) and Klimka *et al.*, Br. J. Cancer, 83:252-260 (2000) (describing the "guided selection" approach to FR shuffling).

**[0175]** Human framework regions that may be used for humanization include but are not limited to: framework regions selected using the "best-fit" method (see, *e.g.*, Sims *.et al.* J.

Immunol. 151:2296 (1993)); framework regions derived from the consensus sequence of human antibodies of a particular subgroup of light or heavy chain variable regions (see, *e.g.*, Carter *et al.* Proc. Natl. Acad. Sci. USA, 89:4285 (1992); and Presta *et al.* J. Immunol., 151:2623 (1993)); human mature (somatically mutated) framework regions or human germline framework regions (see, *e.g.*, Almagro and Fransson, Front. Biosci. 13:1619-1633 (2008)); and framework regions derived from screening FR libraries (see, *e.g.*, Baca *et al.*, J. Biol. Chem. 272:10678-10684 (1997) and Rosok *et al.*, J. Biol. Chem. 271:22611-22618 (1996)).

***4. Human Antibodies***

**[0176]** In certain embodiments, an antibody provided herein is a human antibody. Human antibodies can be produced using various techniques known in the art. Human antibodies are described generally in van Dijk and van de Winkel, Curr. Opin. Pharmacol. 5: 368-74 (2001) and Lonberg, Curr. Opin. Immunol. 20:450-459 (2008).

**[0177]** Human antibodies may be prepared by administering an immunogen to a transgenic animal that has been modified to produce intact human antibodies or intact antibodies with human variable regions in response to antigenic challenge. Such animals typically contain all or a portion of the human immunoglobulin loci, which replace the endogenous immunoglobulin loci, or which are present extrachromosomally or integrated randomly into the animal's chromosomes. In such transgenic mice, the endogenous immunoglobulin loci have generally been inactivated. For review of methods for obtaining human antibodies from transgenic animals, see Lonberg, Nat. Biotech. 23:1117-1125 (2005). *See also, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 describing XENOMOUSE™ technology; U.S. Patent No. 5,770,429 describing HUMAB® technology; U.S. Patent No. 7,041,870 describing K-M MOUSE® technology, and U.S. Patent Application Publication No. US 2007/0061900, describing VELOCIMOUSE® technology). Human variable regions from intact antibodies generated by such animals may be further modified, *e.g.*, by combining with a different human constant region.

**[0178]** Human antibodies can also be made by hybridoma-based methods. Human myeloma and mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described. (See, *e.g.*, Kozbor J. Immunol., 133: 3001 (1984); Brodeur *et al.*, Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner *et al.*, J. Immunol., 147: 86 (1991).) Human antibodies generated via human B-cell hybridoma technology are also

described in Li *et al.*, Proc. Natl. Acad. Sci. USA, 103:3557-3562 (2006). Additional methods include those described, for example, in U.S. Patent No. 7,189,826 (describing production of monoclonal human IgM antibodies from hybridoma cell lines) and Ni, Xiandai Mianyixue, 26(4):265-268 (2006) (describing human-human hybridomas). Human hybridoma technology (Trioma technology) is also described in Vollmers and Brandlein, Histology and Histopathology, 20(3):927-937 (2005) and Vollmers and Brandlein, Methods and Findings in Experimental and Clinical Pharmacology, 27(3):185-91 (2005).

**[0179]** Human antibodies may also be generated by isolating Fv clone variable domain sequences selected from human-derived phage display libraries. Such variable domain sequences may then be combined with a desired human constant domain. Techniques for selecting human antibodies from antibody libraries are described below.

***5. Library-Derived Antibodies***

**[0180]** Antibodies of the invention may be isolated by screening combinatorial libraries for antibodies with the desired activity or activities. For example, a variety of methods are known in the art for generating phage display libraries and screening such libraries for antibodies possessing the desired binding characteristics. Such methods are reviewed, *e.g.*, in Hoogenboom *et al.* in Methods in Molecular Biology 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, 2001) and further described, *e.g.*, in the McCafferty *et al.*, Nature 348:552-554; Clackson *et al.*, Nature 352: 624-628 (1991); Marks *et al.*, J. Mol. Biol. 222: 581-597 (1992); Marks and Bradbury, in Methods in Molecular Biology 248:161-175 (Lo, ed., Human Press, Totowa, NJ, 2003); Sidhu *et al.*, J. Mol. Biol. 338(2): 299-310 (2004); Lee *et al.*, J. Mol. Biol. 340(5): 1073-1093 (2004); Fellouse, Proc. Natl. Acad. Sci. USA 101(34): 12467-12472 (2004); and Lee *et al.*, J. Immunol. Methods 284(1-2): 119-132(2004).

**[0181]** In certain phage display methods, repertoires of VH and VL genes are separately cloned by polymerase chain reaction (PCR) and recombined randomly in phage libraries, which can then be screened for antigen-binding phage as described in Winter *et al.*, Ann. Rev. Immunol., 12: 433-455 (1994). Phage typically display antibody fragments, either as single-chain Fv (scFv) fragments or as Fab fragments. Libraries from immunized sources provide high-affinity antibodies to the immunogen without the requirement of constructing hybridomas. Alternatively, the naive repertoire can be cloned (*e.g.*, from human) to provide a single source of antibodies to a wide range of non-self and also self antigens without any immunization as described by Griffiths *et al.*, EMBO J, 12: 725-734 (1993). Finally, naive libraries can also be made synthetically by cloning unrearranged V-gene segments from stem

cells, and using PCR primers containing random sequence to encode the highly variable CDR3 regions and to accomplish rearrangement *in vitro*, as described by Hoogenboom and Winter, J. Mol. Biol., 227: 381-388 (1992). Patent publications describing human antibody phage libraries include, for example: US Patent No. 5,750,373, and US Patent Publication Nos. 2005/0079574, 2005/0119455, 2005/0266000, 2007/0117126, 2007/0160598, 2007/0237764, 2007/0292936, and 2009/0002360.

**[0182]** Antibodies or antibody fragments isolated from human antibody libraries are considered human antibodies or human antibody fragments herein.

***6. Multispecific Antibodies***

**[0183]** In certain embodiments, an antibody provided herein is a multispecific antibody, *e.g.* a bispecific antibody. Multispecific antibodies are monoclonal antibodies that have binding specificities for at least two different sites. In certain embodiments, one of the binding specificities is for Abeta and the other is for any other antigen. In certain embodiments, bispecific antibodies may bind to two different epitopes of Abeta. Bispecific antibodies may also be used to localize cytotoxic agents to cells. Bispecific antibodies can be prepared as full length antibodies or antibody fragments.

**[0184]** Techniques for making multispecific antibodies include, but are not limited to, recombinant co-expression of two immunoglobulin heavy chain-light chain pairs having different specificities (see Milstein and Cuello, Nature 305: 537 (1983)), WO 93/08829, and Traunecker *et al.*, EMBO J. 10: 3655 (1991)), and "knob-in-hole" engineering (see, *e.g.*, U.S. Patent No. 5,731,168). Multi-specific antibodies may also be made by engineering electrostatic steering effects for making antibody Fc-heterodimeric molecules (WO 2009/089004A1); cross-linking two or more antibodies or fragments (see, *e.g.*, US Patent No. 4,676,980, and Brennan *et al.*, Science, 229: 81 (1985)); using leucine zippers to produce bi-specific antibodies (see, *e.g.*, Kostelny *et al.*, J. Immunol., 148(5):1547-1553 (1992)); using "diabody" technology for making bispecific antibody fragments (see, *e.g.*, Hollinger *et al.*, Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993)); and using single-chain Fv (sFv) dimers (see,*e.g.* Gruber *et al.*, J. Immunol., 152:5368 (1994)); and preparing trispecific antibodies as described, *e.g.*, in Tutt *et al.* J. Immunol. 147: 60 (1991).

**[0185]** Engineered antibodies with three or more functional antigen binding sites, including "Octopus antibodies," are also included herein (see, *e.g.* US 2006/0025576A1).

**[0186]** The antibody or fragment herein also includes a "Dual Acting FAb" or "DAF" comprising an antigen binding site that binds to Abeta as well as another, different antigen (see, US 2008/0069820, for example).

### *7. Antibody Variants*

**[0187]** In certain embodiments, amino acid sequence variants of the antibodies provided herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of an antibody may be prepared by introducing appropriate modifications into the nucleotide sequence encoding the antibody, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution can be made to arrive at the final construct, provided that the final construct possesses the desired characteristics, *e.g.*, antigen-binding.

**Substitution, Insertion, and Deletion Variants**

**[0188]** In certain embodiments, antibody variants having one or more amino acid substitutions are provided. Sites of interest for substitutional mutagenesis include the HVRs and FRs. Conservative substitutions are shown in Table 1 under the heading of "conservative substitutions." More substantial changes are provided in Table 1 under the heading of "exemplary substitutions," and as further described below in reference to amino acid side chain classes. Amino acid substitutions may be introduced into an antibody of interest and the products screened for a desired activity, *e.g.*, retained/improved antigen binding, decreased immunogenicity, or improved ADCC or CDC.

**TABLE 1**

| Original Residue | Exemplary Substitutions | Conservative Substitutions |
|---|---|---|
| Ala (A) | Val; Leu; Ile | Val |
| Arg (R) | Lys; Gln; Asn | Lys |
| Asn (N) | Gln; His; Asp, Lys; Arg | Gln |
| Asp (D) | Glu; Asn | Glu |
| Cys (C) | Ser; Ala | Ser |
| Gln (Q) | Asn; Glu | Asn |
| Glu (E) | Asp; Gln | Asp |
| Gly (G) | Ala | Ala |

| Original Residue | Exemplary Substitutions | Conservative Substitutions |
|---|---|---|
| His (H) | Asn; Gln; Lys; Arg | Arg |
| Ile (I) | Leu; Val; Met; Ala; Phe; Norleucine | Leu |
| Leu (L) | Norleucine; Ile; Val; Met; Ala; Phe | Ile |
| Lys (K) | Arg; Gln; Asn | Arg |
| Met (M) | Leu; Phe; Ile | Leu |
| Phe (F) | Trp; Leu; Val; Ile; Ala; Tyr | Tyr |
| Pro (P) | Ala | Ala |
| Ser (S) | Thr | Thr |
| Thr (T) | Val; Ser | Ser |
| Trp (W) | Tyr; Phe | Tyr |
| Tyr (Y) | Trp; Phe; Thr; Ser | Phe |
| Val (V) | Ile; Leu; Met; Phe; Ala; Norleucine | Leu |

**[0189]** Amino acids may be grouped according to common side-chain properties:

**[0190]** (1) hydrophobic: Norleucine, Met, Ala, Val, Leu, Ile;

**[0191]** (2) neutral hydrophilic: Cys, Ser, Thr, Asn, Gln;

**[0192]** (3) acidic: Asp, Glu;

**[0193]** (4) basic: His, Lys, Arg;

**[0194]** (5) residues that influence chain orientation: Gly, Pro;

**[0195]** (6) aromatic: Trp, Tyr, Phe.

**[0196]** Non-conservative substitutions will entail exchanging a member of one of these classes for another class.

**[0197]** One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human antibody). Generally, the resulting variant(s) selected for further study will have modifications (*e.g.*, improvements) in certain biological properties (*e.g.*, increased affinity, reduced immunogenicity) relative to the parent antibody and/or will have substantially retained certain biological properties of the parent antibody. An exemplary substitutional variant is an affinity matured antibody. In certain embodiments, affinity matured antibodies will have nanomolar or even picomolar affinities for the target antigen. Affinity matured antibodies are produced by procedures known in the art, including, *e.g.*, using phage display-based affinity maturation techniques such as those described herein. Briefly, one or more HVR residues are mutated and the variant antibodies

displayed on phage and screened for a particular biological activity (*e.g.* binding affinity). Other procedures are also known. Marks *et al.* Bio/Technology 10:779-783 (1992) describes affinity maturation by VH and VL domain shuffling. Random mutagenesis of HVR and/or framework residues is described by: Barbas *et al.* Proc Nat. Acad. Sci, USA 91:3809-3813 (1994); Schier *et al.* Gene 169:147-155 (1996); Yelton *et al.* J. Immunol. 155:1994-2004 (1995); Jackson *et al.,* J. Immunol. 154(7):3310-9 (1995); and Hawkins *et al.* J. Mol. Biol. 226:889-896 (1992).

**[0198]** Alterations (*e.g.*, substitutions) may be made in HVRs, *e.g.*, to improve antibody affinity. Such alterations may be made in HVR "hotspots," *i.e.*, residues encoded by codons that undergo mutation at high frequency during the somatic maturation process (see, *e.g.*, Chowdhury, Methods Mol. Biol. 207:179-196 (2008)), and/or SDRs (a-CDRs), with the resulting variant VH or VL being tested for binding affinity. Affinity maturation by constructing and reselecting from secondary libraries has been described, *e.g.*, in Hoogenboom *et al.* in Methods in Molecular Biology 178:1-37 (O'Brien *et al.*, ed., Human Press, Totowa, NJ, (2001).) In some embodiments of affinity maturation, diversity is introduced into the variable genes chosen for maturation by any of a variety of methods (*e.g.*, error-prone PCR, chain shuffling, or oligonucleotide-directed mutagenesis). A secondary library is then created. The library is then screened to identify any antibody variants with the desired affinity. Another method to introduce diversity involves HVR-directed approaches, in which several HVR residues (*e.g.*, 4-6 residues at a time) are randomized. HVR residues involved in antigen binding may be specifically identified, *e.g.*, using alanine scanning mutagenesis or modeling. CDR-H3 and CDR-L3 in particular are often targeted.

**[0199]** In certain embodiments, substitutions, insertions, or deletions may occur within one or more HVRs so long as such alterations do not substantially reduce the ability of the antibody to bind antigen. For example, conservative alterations (*e.g.*, conservative substitutions as provided herein) that do not substantially reduce binding affinity may be made in HVRs. Such alterations may be outside of HVR "hotspots" or SDRs. In certain embodiments of the variant VH and VL sequences provided above, each HVR either is unaltered, or contains no more than one, two or three amino acid substitutions.

**[0200]** A useful method for identification of residues or regions of an antibody that may be targeted for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells (1989) Science, 244:1081-1085. In this method, a residue or group of target residues (*e.g.*, charged residues such as arg, asp, his, lys, and glu) are identified and replaced by a neutral

or negatively charged amino acid (*e.g.*, alanine or polyalanine) to determine whether the interaction of the antibody with antigen is affected. Further substitutions may be introduced at the amino acid locations demonstrating functional sensitivity to the initial substitutions. Alternatively, or additionally, a crystal structure of an antigen-antibody complex to identify contact points between the antibody and antigen. Such contact residues and neighboring residues may be targeted or eliminated as candidates for substitution. Variants may be screened to determine whether they contain the desired properties.

**[0201]** Amino acid sequence insertions include amino- and/or carboxyl-terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme (*e.g.* for ADEPT) or a polypeptide which increases the serum half-life of the antibody.

**Glycosylation variants**

**[0202]** In certain embodiments, an antibody provided herein is altered to increase or decrease the extent to which the antibody is glycosylated. Addition or deletion of glycosylation sites to an antibody may be conveniently accomplished by altering the amino acid sequence such that one or more glycosylation sites is created or removed.

**[0203]** Where the antibody comprises an Fc region, the carbohydrate attached thereto may be altered. Native antibodies produced by mammalian cells typically comprise a branched, biantennary oligosaccharide that is generally attached by an N-linkage to Asn297 of the CH2 domain of the Fc region. See, *e.g.*, Wright *et al.* TIBTECH 15:26-32 (1997). The oligosaccharide may include various carbohydrates, *e.g.*, mannose, N-acetyl glucosamine (GlcNAc), galactose, and sialic acid, as well as a fucose attached to a GlcNAc in the "stem" of the biantennary oligosaccharide structure. In some embodiments, modifications of the oligosaccharide in an antibody of the invention may be made in order to create antibody variants with certain improved properties.

**[0204]** In one embodiment, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e. g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass

spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, *i.e.*, between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. See, *e.g.*, US Patent Publication Nos. US 2003/0157108 (Presta, L.); US 2004/0093621 (Kyowa Hakko Kogyo Co., Ltd). Examples of publications related to "defucosylated" or "fucose-deficient" antibody variants include: US 2003/0157108; WO 2000/61739; WO 2001/29246; US 2003/0115614; US 2002/0164328; US 2004/0093621; US 2004/0132140; US 2004/0110704; US 2004/0110282; US 2004/0109865; WO 2003/085119; WO 2003/084570; WO 2005/035586; WO 2005/035778; WO2005/053742; WO2002/031140; Okazaki *et al.* J. Mol. Biol. 336:1239-1249 (2004); Yamane-Ohnuki *et al.* Biotech. Bioeng. 87: 614 (2004). Examples of cell lines capable of producing defucosylated antibodies include Lec13 CHO cells deficient in protein fucosylation (Ripka *et al.* Arch. Biochem. Biophys. 249:533-545 (1986); US Pat Appl No US 2003/0157108 A1, Presta, L; and WO 2004/056312 A1, Adams *et al.*, especially at Example 11), and knockout cell lines, such as alpha-1,6-fucosyltransferase gene, FUT8, knockout CHO cells (see, *e.g.*, Yamane-Ohnuki *et al.* Biotech. Bioeng. 87: 614 (2004); Kanda, Y. *et al.*, Biotechnol. Bioeng., 94(4):680-688 (2006); and WO2003/085107).

**[0205]** Antibodies variants are further provided with bisected oligosaccharides, *e.g.*, in which a biantennary oligosaccharide attached to the Fc region of the antibody is bisected by GlcNAc. Such antibody variants may have reduced fucosylation and/or improved ADCC function. Examples of such antibody variants are described, *e.g.*, in WO 2003/011878 (Jean-Mairet *et al.*); US Patent No. 6,602,684 (Umana *et al.*); and US 2005/0123546 (Umana *et al.*). Antibody variants with at least one galactose residue in the oligosaccharide attached to the Fc region are also provided. Such antibody variants may have improved CDC function. Such antibody variants are described, *e.g.*, in WO 1997/30087 (Patel *et al.*); WO 1998/58964 (Raju, S.); and WO 1999/22764 (Raju, S.).

**Fc region variants**

**[0206]** In certain embodiments, one or more amino acid modifications may be introduced into the Fc region of an antibody provided herein, thereby generating an Fc region variant. The Fc region variant may comprise a human Fc region sequence (*e.g.*, a human IgG1, IgG2, IgG3 or IgG4 Fc region) comprising an amino acid modification (*e.g.* a substitution) at one or more amino acid positions.

**[0207]** In certain embodiments, the invention contemplates an antibody variant that possesses some but not all effector functions, which make it a desirable candidate for applications in which the half life of the antibody in vivo is important yet certain effector functions (such as complement and ADCC) are unnecessary or deleterious. *In vitro* and/or in vivo cytotoxicity assays can be conducted to confirm the reduction/depletion of CDC and/or ADCC activities. For example, Fc receptor (FcR) binding assays can be conducted to ensure that the antibody lacks FcγR binding (hence likely lacking ADCC activity), but retains FcRn binding ability. The primary cells for mediating ADCC, NK cells, express FcλRIII only, whereas monocytes express FcλRI, FcλRII and FcλRIII. FcR expression on hematopoietic cells is summarized in Table 3 on page 464 of Ravetch and Kinet, Annu. Rev. Immunol. 9:457-492 (1991). Non-limiting examples of *in vitro* assays to assess ADCC activity of a molecule of interest is described in U.S. Patent No. 5,500,362 (see, *e.g.* Hellstrom, I. *et al.* Proc. Nat'l Acad. Sci. USA 83:7059-7063 (1986)) and Hellstrom, I *et al.*, Proc. Nat'l Acad. Sci. USA 82:1499-1502 (1985); 5,821,337 (see Bruggemann, M. *et al.*, J. Exp. Med. 166:1351-1361 (1987)). Alternatively, non-radioactive assays methods may be employed (see, for example, ACTI™ non-radioactive cytotoxicity assay for flow cytometry (CellTechnology, Inc. Mountain View, CA; and CytoTox 96® non-radioactive cytotoxicity assay (Promega, Madison, WI). Useful effector cells for such assays include peripheral blood mononuclear cells (PBMC) and Natural Killer (NK) cells. Alternatively, or additionally, ADCC activity of the molecule of interest may be assessed in vivo, *e.g.*, in a animal model such as that disclosed in Clynes *et al.* Proc. Nat'l Acad. Sci. USA 95:652-656 (1998). C1q binding assays may also be carried out to confirm that the antibody is unable to bind C1q and hence lacks CDC activity. See, *e.g.*, C1q and C3c binding ELISA in WO 2006/029879 and WO 2005/100402. To assess complement activation, a CDC assay may be performed (see, for example, Gazzano-Santoro *et al.*, J. Immunol. Methods 202:163 (1996); Cragg, M.S. *et al.*, Blood 101:1045-1052 (2003); and Cragg, M.S. and M.J. Glennie, Blood 103:2738-2743 (2004)). FcRn binding and in vivo clearance/half life determinations can also be performed using methods known in the art (see, *e.g.*, Petkova, S.B. *et al.*, Int'l. Immunol. 18(12):1759-1769 (2006)).

**[0208]** Antibodies with reduced effector function include those with substitution of one or more of Fc region residues 238, 265, 269, 270, 297, 327 and 329 (U.S. Patent No. 6,737,056). Such Fc mutants include Fc mutants with substitutions at two or more of amino acid positions 265, 269, 270, 297 and 327, including the so-called "DANA" Fc mutant with substitution of residues 265 and 297 to alanine (US Patent No. 7,332,581).

**[0209]** Certain antibody variants with improved or diminished binding to FcRs are described. (See, *e.g.*, U.S. Patent No. 6,737,056; WO 2004/056312, and Shields *et al.*, J. Biol. Chem. 9(2): 6591-6604 (2001).)

**[0210]** In certain embodiments, an antibody variant comprises an Fc region with one or more amino acid substitutions which improve ADCC, *e.g.*, substitutions at positions 298, 333, and/or 334 of the Fc region (EU numbering of residues).

**[0211]** In some embodiments, alterations are made in the Fc region that result in altered (*i.e.*, either improved or diminished) C1q binding and/or Complement Dependent Cytotoxicity (CDC), *e.g.*, as described in US Patent No. 6,194,551, WO 99/51642, and Idusogie *et al.* J. Immunol. 164: 4178-4184 (2000).

**[0212]** Antibodies with increased half lives and improved binding to the neonatal Fc receptor (FcRn), which is responsible for the transfer of maternal IgGs to the fetus (Guyer *et al.*, J. Immunol. 117:587 (1976) and Kim *et al.*, J. Immunol. 24:249 (1994)), are described in US2005/0014934A1 (Hinton *et al.*). Those antibodies comprise an Fc region with one or more substitutions therein which improve binding of the Fc region to FcRn. Such Fc variants include those with substitutions at one or more of Fc region residues: 238, 256, 265, 272, 286, 303, 305, 307, 311, 312, 317, 340, 356, 360, 362, 376, 378, 380, 382, 413, 424 or 434, *e.g.*, substitution of Fc region residue 434 (US Patent No. 7,371,826). See also Duncan & Winter, Nature 322:738-40 (1988); U.S. Patent No. 5,648,260; U.S. Patent No. 5,624,821; and WO 94/29351 concerning other examples of Fc region variants.

**Cysteine engineered antibody variants**

**[0213]** In certain embodiments, it may be desirable to create cysteine engineered antibodies, *e.g.*, "thioMAbs," in which one or more residues of an antibody are substituted with cysteine residues. In particular embodiments, the substituted residues occur at accessible sites of the antibody. By substituting those residues with cysteine, reactive thiol groups are thereby positioned at accessible sites of the antibody and may be used to conjugate the antibody to other moieties, such as drug moieties or linker-drug moieties, to create an immunoconjugate, as described further herein. In certain embodiments, any one or more of the following residues may be substituted with cysteine: V205 (Kabat numbering) of the light chain; A118 (EU numbering) of the heavy chain; and S400 (EU numbering) of the heavy chain Fc region. Cysteine engineered antibodies may be generated as described, *e.g.*, in U.S. Patent No. 7,521,541.

**Antibody Derivatives**

**[0214]** In certain embodiments, an antibody provided herein may be further modified to contain additional nonproteinaceous moieties that are known in the art and readily available. The moieties suitable for derivatization of the antibody include but are not limited to water soluble polymers. Non-limiting examples of water soluble polymers include, but are not limited to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, propropylene glycol homopolymers, prolypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, etc.

**[0215]** In another embodiment, conjugates of an antibody and nonproteinaceous moiety that may be selectively heated by exposure to radiation are provided. In one embodiment, the nonproteinaceous moiety is a carbon nanotube (Kam *et al.*, Proc. Natl. Acad. Sci. USA 102: 11600-11605 (2005)). The radiation may be of any wavelength, and includes, but is not limited to, wavelengths that do not harm ordinary cells, but which heat the nonproteinaceous moiety to a temperature at which cells proximal to the antibody-nonproteinaceous moiety are killed.

**Recombinant Methods and Compositions**

**[0216]** Antibodies may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In one embodiment, isolated nucleic acid encoding an anti-Abeta antibody described herein is provided. Such nucleic acid may encode an amino acid sequence comprising the VL and/or an amino acid sequence comprising the VH of the antibody (*e.g.*, the light and/or heavy chains of the antibody). In a further embodiment, one or more vectors (*e.g.*, expression vectors) comprising such nucleic acid are provided. In a further embodiment, a host cell comprising such nucleic acid is provided. In one such embodiment, a host cell comprises (*e.g.*, has been transformed with): (1) a vector comprising a nucleic acid that

encodes an amino acid sequence comprising the VL of the antibody and an amino acid sequence comprising the VH of the antibody, or (2) a first vector comprising a nucleic acid that encodes an amino acid sequence comprising the VL of the antibody and a second vector comprising a nucleic acid that encodes an amino acid sequence comprising the VH of the antibody. In one embodiment, the host cell is eukaryotic, *e.g.* a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell). In one embodiment, a method of making an anti-Abeta antibody is provided, wherein the method comprises culturing a host cell comprising a nucleic acid encoding the antibody, as provided above, under conditions suitable for expression of the antibody, and optionally recovering the antibody from the host cell (or host cell culture medium).

**[0217]** For recombinant production of an anti-Abeta antibody, nucleic acid encoding an antibody, *e.g.*, as described above, is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

**[0218]** Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells described herein. For example, antibodies may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria, see, *e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523. (See also Charlton, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*.) After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

**[0219]** In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been "humanized," resulting in the production of an antibody with a partially or fully human glycosylation pattern. See Gerngross, Nat. Biotech. 22:1409-1414 (2004), and Li *et al.*, Nat. Biotech. 24:210-215 (2006).

**[0220]** Suitable host cells for the expression of glycosylated antibody are also derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of Spodoptera frugiperda cells.

**[0221]** Plant cell cultures can also be utilized as hosts. *See, e.g.*, US Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429 (describing PLANTIBODIES™ technology for producing antibodies in transgenic plants).

**[0222]** Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham *et al.*, J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor (MMT 060562); TRI cells, as described, *e.g.*, in Mather *et al.*, Annals N.Y. Acad. Sci. 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub *et al.*, Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, *e.g.*, Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

**Assays**

**[0223]** Anti-Abeta antibodies provided herein may be identified, screened for, or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

***Binding assays and other assays***

**[0224]** In one aspect, an antibody of the invention is tested for its antigen binding activity, *e.g.*, by known methods such as ELISA, Western blot, etc.

**[0225]** In another aspect, competition assays may be used to identify an antibody that competes with an anti-Abeta antibody of the invention for binding to Abeta. In certain embodiments, such a competing antibody binds to the same epitope (*e.g.*, a linear or a conformational epitope) that is bound by crenezumab or another anti-Abeta antibody specified herein. Detailed exemplary methods for mapping an epitope to which an antibody binds are provided in Morris (1996) "Epitope Mapping Protocols," in Methods in Molecular Biology vol. 66 (Humana Press, Totowa, NJ).

**[0226]** In an exemplary competition assay, immobilized Abeta in the desired form (*e.g.*, monomeric, oligomeric, or fibril) is incubated in a solution comprising a first labeled antibody that binds to Abeta (*e.g.*, crenezumab) and a second unlabeled antibody that is being tested for its ability to compete with the first antibody for binding to Abeta. The second antibody may be present in a hybridoma supernatant. As a control, immobilized Abeta is incubated in a solution comprising the first labeled antibody but not the second unlabeled antibody. After incubation under conditions permissive for binding of the first antibody to Abeta, excess unbound antibody is removed, and the amount of label associated with immobilized Abeta is measured. If the amount of label associated with immobilized Abeta is substantially reduced in the test sample relative to the control sample, then that indicates that the second antibody is competing with the first antibody for binding to Abeta. See Harlow and Lane (1988) Antibodies: A Laboratory Manual ch.14 (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).

***Activity assays***

**[0227]** In one aspect, assays are provided for identifying anti-Abeta antibodies thereof having biological activity, for example the biological activity of crenezumab. Biological activity may include, but is not limited to, *e.g.*, prevention of aggregation of monomeric Abeta into oligomeric Abeta, or disaggregation of oligomeric Abeta into monomeric Abeta. Antibodies having such biological activity in vivo and/or *in vitro* are also provided.

**[0228]** In certain embodiments, an antibody of the invention is tested for such biological activity.

**Methods and Compositions for Diagnostics and Detection**

**[0229]** In certain embodiments, any of the anti-Abeta antibodies provided herein is useful for detecting the presence of Abeta in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue, such as serum, plasma, nasal swabs, sputum, cerebrospinal fluid, , aqueous humor of the eye and the like, or tissue or cell samples obtained from an organism such as samples containing neural or brain tissue.

**[0230]** In one embodiment, an anti-Abeta antibody for use in a method of diagnosis or detection is provided. In a further aspect, a method of detecting the presence of Abeta in a biological sample is provided. In certain embodiments, the method comprises contacting the biological sample with an anti-Abeta antibody as described herein under conditions permissive for binding of the anti-Abeta antibody to Abeta, and detecting whether a complex is formed between the anti-Abeta antibody and Abeta. Such method may be an *in vitro* or in vivo method.

**[0231]** Exemplary disorders that may be diagnosed using an antibody of the invention are diseases and disorders caused by or associated with amyloid or amyloid-like proteins. These include, but are not limited to, diseases and disorders caused by the presence or activity of amyloid-like proteins in monomeric, fibril, or polymeric state, or any combination of the three, including by amyloid plaques. Exemplary diseases include, but are not limited to, secondary amyloidosis and age-related amyloidosis, such as diseases including, but not limited to, neurological disorders such as Alzheimer's Disease ("AD"), diseases or conditions characterized by a loss of cognitive memory capacity such as, for example, mild cognitive impairment (MCI), Lewy body dementia, Down's syndrome, hereditary cerebral hemorrhage with amyloidosis (Dutch type), the Guam Parkinson-Demential complex and other diseases which are based on or associated with amyloid-like proteins such as progressive supranuclear palsy, multiple sclerosis, Creutzfeld Jacob disease, Parkinson's disease, HIV-related dementia, ALS (amyotropic lateral sclerosis), inclusion-body myositis (IBM), adult onset diabetes, endocrine tumor and senile cardiac amyloidosis, and various eye diseases including macular degeneration, drusen-related optic neuropathy, glaucoma, and cataract due to beta-amyloid deposition.

**[0232]** In certain embodiments, labeled anti-Abeta antibodies are provided. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction. Exemplary labels include, but are not limited to, the radioisotopes $^{32}P$, $^{14}C$, $^{125}I$, $^{3}H$, and $^{131}I$, fluorophores such as rare earth chelates or fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, luceriferases, *e.g.*, firefly luciferase and bacterial luciferase (U.S. Patent No. 4,737,456), luciferin, 2,3-dihydrophthalazinediones, horseradish peroxidase (HRP), alkaline phosphatase, β-galactosidase, glucoamylase, lysozyme, saccharide oxidases, *e.g.*, glucose oxidase, galactose oxidase, and glucose-6-phosphate dehydrogenase, heterocyclic oxidases such as uricase and xanthine oxidase, coupled with an enzyme that employs hydrogen peroxide to oxidize a dye precursor such as HRP, lactoperoxidase, or microperoxidase, biotin/avidin, spin labels, bacteriophage labels, stable free radicals, and the like.

**Pharmaceutical Formulations**

**[0233]** Pharmaceutical formulations of an anti-Abeta antibody as described herein are prepared by mixing such antibody or molecule having the desired degree of purity with one or

more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or aqueous solutions. Pharmaceutically acceptable carriers are generally nontoxic to recipients at the dosages and concentrations employed, and include, but are not limited to: buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (*e.g.* Zn-protein complexes); and/or non-ionic surfactants such as polyethylene glycol (PEG). Exemplary pharmaceutically acceptable carriers herein further include insterstitial drug dispersion agents such as soluble neutral-active hyaluronidase glycoproteins (sHASEGP), for example, human soluble PH-20 hyaluronidase glycoproteins, such as rHuPH20 (HYLENEX®, Baxter International, Inc.). Certain exemplary sHASEGPs and methods of use, including rHuPH20, are described in US Patent Publication Nos. 2005/0260186 and 2006/0104968. In one aspect, a sHASEGP is combined with one or more additional glycosaminoglycanases such as chondroitinases.

**[0234]** In one embodiment, an antibody of the invention may be formulated in an arginine buffer. In one aspect, the arginine buffer may be an arginine succinate buffer. In one such aspect, the concentration of the arginine succinate buffer may be 50 mM or greater. In another such aspect, the concentration of the arginine succinate buffer may be 100 mM or greater. In another such aspect, the concentration of the arginine succinate buffer may be 150 mM or greater. In another such aspect, the concentration of the arginine succinate buffer may be 200 mM or greater. In another aspect, the arginine buffer formulation may further contain a surfactant. In another such aspect, the surfactant is a polysorbate. In another such aspect, the polysorbate is polysorbate 20. In another such aspect, the concentration of polysorbate 20 in the formulation is 0.1% or less. In another such aspect, the concentration of polysorbate 20 in the formulation is 0.05% or less. In another aspect, the pH of the arginine buffer formulation is between 4.5 and 7.0. In another aspect, the pH of the arginine buffer formulation is between 5.0

and 6.5. In another aspect, the pH of the arginine buffer formulation is between 5.0 and 6.0. In another aspect, the pH of the arginine buffer formulation is 5.5. In any of the foregoing embodiments and aspects, the antibody of the invention may be crenezumab.

**[0235]** Exemplary lyophilized antibody formulations are described in US Patent No. 6,267,958. Aqueous antibody formulations include those described in US Patent No. 6,171,586 and WO2006/044908, the latter formulations including a histidine-acetate buffer.

**[0236]** The formulation herein may also contain more than one active ingredients as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be desirable to further provide one or more compounds to prevent or treat symptoms of Alzheimer's Disease. Such active ingredients are suitably present in combination in amounts that are effective for the purpose intended.

**[0237]** Active ingredients may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization, for example, hydroxymethylcellulose or gelatin-microcapsules and poly-(methylmethacylate) microcapsules, respectively, in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules) or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980).

**[0238]** Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, *e.g.* films, or microcapsules.

**[0239]** The formulations to be used for in vivo administration are generally sterile. Sterility may be readily accomplished, *e.g.*, by filtration through sterile filtration membranes.

**Therapeutic Methods and Compositions**

**[0240]** As shown herein, intravenous administration of crenezumab reduced disease progression in patients suffering from AD. Specifically, patients with mild to moderate AD, including patients with mild AD and ApoE4 positive patients, as well as patients with brain amyloid load typically seen in patients diagnosed with AD, showed a reduction in the rate of cognitive decline when treated with crenezumab as compared to a placebo. The milder the disease, based on increasing MMSE score, the greater the reduction in decline in the treatment arm as compared to the placebo arm. These results were further substantiated by other indications of target engagement by crenezumab, including an increase in the levels of Abeta detected in cerebrospinal fluid and reduction in the accumulation of amyloid in the brain.

Furthermore, a comparatively high dose of antibody – 15 mg/kg – did not increase the incidence of the ARIA-type adverse events which have been observed in trials of other anti-Abeta antibodies.

**[0241]** Therefore, in one embodiment, an antibody of the invention is used to treat AD, including mild to moderate AD, mild AD, and early AD. In another embodiment, an antibody of the invention is used to treat an amyloidosis. In one such embodiment, the amyloidosis is mild cognitive impairment. In another such embodiment, the amyloidosis is Down's syndrome. In another such embodiment, the amyloidosis is hereditary cerebral hemorrhage with amyloidosis (Dutch type). In another such embodiment, the amyloidosis is the Guam Parkinson-Dementia complex. In another such embodiment, the amyloidosis is an ocular disease related to drusen or other amyloid deposit in the eye. In one aspect, the ocular disease is macular degeneration. In another aspect, the ocular disease is a drusen-related optic neuropathy. In another aspect, the ocular disease is glaucoma. In another aspect, the ocular disease is cataract. In any of the foregoing embodiments and aspects, the antibody of the invention may be crenezumab.

**[0242]** A patient is typically first assessed for the presence of one or more amyloidosis prior to determining the suitability of an antibody of the invention to treat such patient. As one nonlimiting example, AD may be diagnosed in a patient using the "NINCDS-ADRDA" (Neurological and Communicative Disorders and Stroke-Alzheimer's Disease Related Disorders Assessment) criteria. *See* McKhann, *et al.*, 1984, Neurology 34:939–44. A potential patient to be administered one or more antibodies of the invention may also be tested for the presence or absence of one or more genetic markers which may predispose such patient either to (i) a higher or lower likelihood of such patient experiencing one or more amyloidoses, or (ii) a higher or lower likelihood of such patient experiencing one or more adverse events or side effects during the course of administration of an antibody of the invention. As one nonlimiting example, it is known that patients carrying the ApoE4 allele have a substantially higher risk of developing AD than those lacking the allele (Saunders *et al.*, Neurology 1993; 43:1467-72; Prekumar *et al.*, Am. J. Pathol. 1996; 148:2083-95), and that such patients were disproportionately represented in ARIA-type adverse events observed in the clinical trial of bapineuzumab, another anti-Abeta antibody (Sperling *et al.*, Alzheimer's & Dementia 2011, 7:367-385; Salloway *et al.*, N. Engl. J. Med. 2014, 370:322-333).

**[0243]** In some embodiments, the antibody of the invention is used to treat mild to moderate AD in a patient. The patient can be ApoE4 positive or ApoE4 negative. In some

embodiments, the antibody of the invention is used to treat mild AD. In some embodiments, the antibody of the invention is used to treat an ApoE4 positive patient suffering from mild to moderate AD or mild AD. In some embodiments, the antibody of the invention is used to treat a patient suffering from mild AD.

**[0244]** In some embodiments, the antibody of the invention is used to treat a patient having an MMSE score of between 20 and 30, between 20 and 26, between 24 and 30, between 21and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26. In some embodiments, the patient has an MMSE score between 22 and 26. As used herein, an MMSE score between two numbers includes the numbers at each end of the range. For example, an MMSE score between 22 and 26 includes MMSE scores of 22 and 26.

**[0245]** In some embodiments, the antibodies of the invention are used to treat a patient who is 'amyloid positive,' *e.g.*, a patient having brain amyloid deposits that are typical of a patient diagnosed with AD or a patient having a positive florbetapir PET scan. In some embodiments, the antibodies of the invention are used to reduce the accumulation of brain amyloid deposits or neuritic plaques (*i.e.*, to reduce an increase in brain amyloid burden or load).

**[0246]** Furthermore, the antibodies of the invention are useful for treating mild to moderate AD without increasing the incidence of ARIA-E or ARIA-H. In some embodiments, the patients are suffering from mild AD. In some embodiments, the patients are ApoE4 positive. In some embodiments, the patients are ApoE4 positive and suffering from mild AD.

**[0247]** As evidenced in the Examples herein, the therapeutic effect is increased in patients with milder forms of AD. Consequently, in some embodiments, the antibody of the invention is used to treat a patient with early AD. In certain embodiments, the patient to be treated has one or more of the following characteristics: (a) mild cognitive impairement (MCI) due to AD; (b) one or more biomarkers indicative of Alzheimer's Disease without a clinically detectable deficit; (c) an objective memory loss quantified using the Free and Cued Selective Reminding Test (FCSRT) as a score of 27 or greater; an MMSE of 24-30; (d) a global Clinical Dementia Rating (CDR) of 0.5; and (e) a positive amyloid PET scan (as determined by a qualified reader).

**[0248]** Antibodies of the invention are formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual subject, the cause of the disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

**Routes of Administration**

**[0249]** An antibody of the invention (and any additional therapeutic agent) can be administered by any suitable means, including parenteral, intrapulmonary, and intranasal, and, if desired for local treatment, intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. Dosing can be by any suitable route, *e.g.* by injections, such as intravenous or subcutaneous injections, depending in part on whether the administration is brief or chronic. In one embodiment, the antibody is injected subcutaneously. In another embodiment, the antibody is injected intravenously. In another embodiment, the antibody is administered using a syringe (*e.g.*, prefilled or not) or an autoinjector. In another embodiment, the antibody is inhaled.

**Dosing**

**[0250]** For the treatment of an amyloidosis, the appropriate dosage of an antibody of the invention (when used alone or in combination with one or more other additional therapeutic agents) will depend on the specific type of disease to be treated, the type of antibody, the severity and course of the disease, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The antibody is suitably administered to the patient at one time or over a series of treatments. Various dosing schedules including, but not limited to, single or multiple administrations over various time-points, bolus administration, and pulse infusion are contemplated herein.

**[0251]** Depending on the type and severity of the disease, about 0.3 mg/kg to 100 mg/kg (*e.g.* 15 mg/kg-100 mg/kg, or any dosage within that range) of antibody can be an initial candidate dosage for administration to the patient, whether, for example, by one or more separate administrations, or by continuous infusion. One typical daily dosage might range from about 15 mg/kg to 100 mg/kg or more, depending on the factors mentioned above. The dosage can be administered in a single dose or a divided dose (e.g., two doses of 15 mg/kg for a total dose of 30 mg/ kg). For repeated administrations over several weeks or longer, depending on the condition, the treatment would generally be sustained until a desired suppression of disease symptoms occurs. One exemplary dosage of the antibody would be in the range from about 10 mg/kg to about 50 mg/kg. Thus, one or more doses of about 0.5 mg/kg, 1 mg/kg, 1.5 mg/kg, 2.0 mg/kg, 3 mg/kg, 4.0 mg/kg, 5 mg/kg, 10 mg/kg, 15 mg/kg, 20 mg/kg, 25 mg/kg, 30 mg/kg, 35 mg/kg, 40 mg/kg, 50 mg/ kg, 60 mg/ kg, 70 mg/kg, 80 mg/kg, 90 mg/kg, or 100 mg/kg (or any combination thereof) may be administered to the patient. In some embodiments, the total dose administered is in the range of 50 mg to 2500 mg. An exemplary dose of about 50 mg, about

100 mg, 200 mg, 300 mg, 400 mg, about 500 mg, about 600 mg, about 700 mg, about 720 mg, about 1000 mg, about 1050 mg, about 1100 mg, about 1200 mg, about 1300 mg, about 1400 mg, about 1500 mg, about 1600 mg, about 1700 mg, about 1800 mg, about 1900 mg, about 2000 mg, about 2050 mg, about 2100 mg, about 2200 mg, about 2300 mg, about 2400 mg, or about 2500 mg (or any combination thereof) may be administered to the patient. Such doses may be administered intermittently, *e.g.* every week, every two weeks, every three weeks, every four weeks, every month, every two months, every three months, or every six months. In some embodiments, the patient receives from one to thirty five doses (*e.g.* about eighteen doses of the antibody). However, other dosage regimens may be useful. The progress of this therapy can be monitored by conventional techniques and assays.

**[0252]** In certain embodiments, an antibody of the invention is administered at a dose of 15 mg/kg, 30 mg/kg, 40 mg/kg, 45 mg/kg, 50 mg/kg, 60 mg/kg or a flat dose, e.g., 300 mg, 500 mg, 700 mg, 800 mg, or higher. In some embodiments, the dose is administered by intravenous injection every 2 weeks or every 4 weeks for a period of time. In some embodiments, the dose is administered by subcutaneous injection every 2 weeks or every 4 weeks for a period of time. In certain embodiments, the period of time is 6 months, one year, eighteen months, two years, five years, ten years, 15 years, 20 years, or the lifetime of the patient.

**Monitoring/Assessing Response to Therapeutic Treatment**

**[0253]** As used in methods of the present disclosure, the antibody, or antigen-binding fragment hereof, provides therapeutic effect or benefit to the patient. In certain embodiments, the therapeutic benefit is a delay in, or inihibition of, progression of AD or a reduction in clinical, functional, or cognitive decline. In some embodiments, therapeutic effect or benefit is reflected in a "patient response" or "response" (and grammatical variations thereof). Patient response can be assessed using any endpoint indicating a benefit to the patient, including, without limitation, (1) inhibition, to some extent, of disease progression, including slowing down and complete arrest; (2) reduction in amount of plaque or reduction in brain amyloid accumulation; (3) improvement in one or more assessment metrics, including but not limited to ADAS-Cog, iADL, and CDR-SOB scales; (4) improvement in daily functioning of the patient; (5) increase in concentration of one or more biomarkers, *e.g.*, Abeta, in cerebrospinal fluid; and (6) decrease in one or more biomarkers indicative of the presence of AD. An assessment of patient response may also include an assessment of any adverse events that may occur that may be correlated with the treatment.

**[0254]** In one embodiment, the cognitive ability and daily functioning of the patient is assessed prior to, during, and/or after a course of therapy with an antibody of the invention. A number of cognitive and functional assessment tools have been developed for use in assessing, diagnosing, and scoring mental function, cognition, and neurological deficit. These tools include, but are not limited to, the ADAS-Cog, including the 12 item ADAS-Cog (ADAS-Cog12), the 13-item ADAS-Cog (ADAS-Cog13), the 14-item ADAS-Cog (ADAS-Cog14); the CDR-SOB, including CDR Judgment and Problem solving and CDR Memory components; the Instrumental Activities of Daily Living (iADL); and the MMSE.

**[0255]** "ADAS-Cog" refers to the Alzheimer's Disease Assessment Scale Cognitive Subscale, a multi-part cognitive assessment. *See* Rosen *et al.*, 1984, Amer. J. Psych. 141:1356-1364; Mohs *et al.*, 1997, Alzheimer's Disease Assoc. Disorders 11(2):S13-S21. The higher the numerical score on the ADAS-Cog, the greater the tested patient's deficit or impairment relative to another individual with a lower score. The ADAS-Cog may be used as one measure for assessing whether a treatment for AD is therapeutically effective. An increase in ADAS-Cog score is indicative of worsening in the patient's condition, whereas a decrease in ADAS-Cog score denotes improvement in the patient's condition. As used herein, a "decline in ADAS-Cog performance" or an "increase in ADAS-Cog score" indicates a worsening in the patient's condition and may reflect progression of AD. The ADAS-Cog is an examiner-administered battery that assesses multiple cognitive domains, including memory, comprehension, praxis, orientation, and spontaneous speech (Rosen et al. 1984, Am J Psychiatr 141:1356−64; Mohs et al. 1997, Alzheimer Dis Assoc Disord 11(S2):S13−S21). The ADAS-Cog is a standard primary endpoint in AD treatment trials (Mani 2004, Stat Med 23:305−14). The ADAS-Cog12 is the 70-point version of the ADAS-Cog plus a 10-point Delayed Word Recall item assessing recall of a learned word list. Other ADAS-Cog scales include the ADAS-Cog13 and ADAS-Cog14.

**[0256]** In some embodiments, the methods of treatment provided herein provide a reduction in cognitive decline as measured by an ADAS-Cog score that is at least about 30%, at least about 35%, at least about 40%, or at least about 45% lower relative to placebo.

**[0257]** "MMSE" refers to the Mini Mental State Examination, which provides a score between 1 and 30. *See* Folstein, *et al.*, 1975, J. Psychiatr. Res. 12:189–98. Scores of 26 and lower are generally considered to be indicative of a deficit. The lower the numerical score on the MMSE, the greater the tested patient's deficit or impairment relative to another individual with a lower score. An increase in MMSE score may be indicative of improvement in the

patient's condition, whereas a decrease in MMSE score may denote worsening in the patient's condition.

**[0258]** "CDR-SOB" refers to the Clinical Dementia Rating Scale / Sum of Boxes. *See* Hughes *et al*, 1982. CDR-assesses 6 components: memory, orientation, judgment/problem solving, community affairs, home and hobbies, and personal care. The test is administered to both the patient and the caregiver and each component (or each "box"), is scored on a scale of 0 to 3. A complete CDR-SOB score is based on the sum of the scores across all 6 boxes. Subscores can be obtained for each of the boxes or components individually as well, *e.g.*, CDR/ Memory or CDR/ Judgment and Problem solving. As used herein, a "decline in CDR-SOB performance" or an "increase in CDR-SOB score" indicates a worsening in the patient's condition and may reflect progression of AD. In some embodiments, the methods of treatment provided herein provide a reduction in decline in CDR-SOB performance of at least about 30%, at least about 35%, or at least about 40% relative to placebo.

**[0259]** "iADL" refers to the Instrumental Activities of Daily Living scale. *See* Lawton, M.P., and Brody, E.M., 1969, Gerontologist 9:179-186. This scale measures the ability to perform typical daily activities such as housekeeping, laundry, operating a telephone, shopping, preparing meals, etc. The lower the score, the more impaired the individual is in conducting activities of daily living. In some embodiments, the methods of treatment provided herein provide a reduction in decline of at least about 10%, at least about 15%, or at least about 20% on the iADL scale relative to placebo.

**[0260]** Brain amyloid load or burden can be determined using neurological imaging techniques and tools, for example using PET (positron emission tomography) scanning. Serial PET scans of a patient taken over time, *e.g.*, before and after administration of a treatment (or at one or more intervals throughout the course of a treatment regimen), can permit detection of increased, decreased, or unchanged amyloid burden in the brain. This technique can further be used to determine whether amyloid accumulation is increasing or decreasing. In some embodiments, detection of amyloid deposits in the brain is performed using florbetapir $^{18}$F. In some embodiments, a florbetapir PET scan is considered positive if, based on a centralized visual read of the scan, it establishes the presence of moderate-to-frequent neuritic plaques.

**Co-Administration**

**[0261]** The antibody need not be, but is optionally formulated with one or more agents currently used to prevent or treat the disorder in question or one or more of its symptoms. The

effective amount of such other agents depends on the amount of antibody present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in the same dosages and with administration routes as described herein, or about from 1 to 99% of the dosages described herein, or in any dosage and by any route that is empirically/clinically determined to be appropriate. It will be understood by one of ordinary skill in the art that an antibody of the invention may be co-administered simultaneously with any of the foregoing compounds, or may be administered prior to or subsequent to administration of any of the foregoing compounds.

**[0262]** When treating an amyloidosis with an antibody of the invention, a neurological drug may be co-administered. Such neurological drug may be selected from the group including, but not limited to, an antibody or other binding molecule (including, but not limited to a small molecule, a peptide, an aptamer, or other protein binder) that specifically binds to a target selected from: beta secretase, tau, presenilin, amyloid precursor protein or portions thereof, amyloid beta peptide or oligomers or fibrils thereof, death receptor 6 (DR6), receptor for advanced glycation endproducts (RAGE), parkin, and huntingtin; a cholinesterase inhibitor (*i.e.*, galantamine, donepezil, rivastigmine and tacrine); an NMDA receptor antagonist (*i.e.*, memantine), a monoamine depletor (*i.e.*, tetrabenazine); an ergoloid mesylate; an anticholinergic antiparkinsonism agent (*i.e.*, procyclidine, diphenhydramine, trihexylphenidyl, benztropine, biperiden and trihexyphenidyl); a dopaminergic antiparkinsonism agent (*i.e.*, entacapone, selegiline, pramipexole, bromocriptine, rotigotine, selegiline, ropinirole, rasagiline, apomorphine, carbidopa, levodopa, pergolide, tolcapone and amantadine); a tetrabenazine; an anti-inflammatory (including, but not limited to, a nonsteroidal anti-inflammatory drug (*i.e.*, indomethicin and other compounds listed above); a hormone (*i.e.*, estrogen, progesterone and leuprolide); a vitamin (*i.e.*, folate and nicotinamide); a dimebolin; a homotaurine (*i.e.*, 3-aminopropanesulfonic acid; 3APS); a serotonin receptor activity modulator (*i.e.*, xaliproden); an, an interferon, and a glucocorticoid or corticosteroid. In some embodiments, one or more anti-Abeta antibodies other than crenezumab are co-administered. Non-limiting examples of such anti-Abeta antibodies include solanezumab, bapineuzumab, aducanumab, and gantenerumab. The term "corticosteroid" includes, but is not limited to fluticasone (including fluticasone propionate (FP)), beclometasone, budesonide, ciclesonide, mometasone, flunisolide, betamethasone and triamcinolone. "Inhalable corticosteroid" means a corticosteroid that is suitable for delivery by inhalation. Exemplary inhalable corticosteroids are fluticasone, beclomethasone

dipropionate, budenoside, mometasone furoate, ciclesonide, flunisolide, and triamcinolone acetonide.

**[0263]** When treating an amyloidosis that is an ocular disease or disorder with an antibody of the invention, a neurological drug may be selected that is an anti-angiogenic ophthalmic agent (*i.e.*, bevacizumab, ranibizumab and pegaptanib), an ophthalmic glaucoma agent (*i.e.*, carbachol, epinephrine, demecarium bromide, apraclonidine, brimonidine, brinzolamide, levobunolol, timolol, betaxolol, dorzolamide, bimatoprost, carteolol, metipranolol, dipivefrin, travoprost and latanoprost), a carbonic anhydrase inhibitor (*i.e.*, methazolamide and acetazolamide), an ophthalmic antihistamine (*i.e.*, naphazoline, phenylephrine and tetrahydrozoline), an ocular lubricant, an ophthalmic steroid (*i.e.*, fluorometholone, prednisolone, loteprednol, dexamethasone, difluprednate, rimexolone, fluocinolone, medrysone and triamcinolone), an ophthalmic anesthetic (*i.e.*, lidocaine, proparacaine and tetracaine), an ophthalmic anti-infective (*i.e.*, levofloxacin, gatifloxacin, ciprofloxacin, moxifloxacin, chloramphenicol, bacitracin/polymyxin b, sulfacetamide, tobramycin, azithromycin, besifloxacin, norfloxacin, sulfisoxazole, gentamicin, idoxuridine, erythromycin, natamycin, gramicidin, neomycin, ofloxacin, trifluridine, ganciclovir, vidarabine), an ophthalmic anti-inflammatory agent (*i.e.*, nepafenac, ketorolac, flurbiprofen, suprofen, cyclosporine, triamcinolone, diclofenac and bromfenac), and an ophthalmic antihistamine or decongestant (*i.e.*, ketotifen, olopatadine, epinastine, naphazoline, cromolyn, tetrahydrozoline, pemirolast, bepotastine, naphazoline, phenylephrine, nedocromil, lodoxamide, phenylephrine, emedastine and azelastine).It is understood that any of the above formulations or therapeutic methods may be carried out using an immunoconjugate of the invention in place of or in addition to an anti-Abeta antibody.

**Articles of Manufacture**

**[0264]** In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one active agent in the

composition is an antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises an antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

**[0265]** It is understood that any of the above articles of manufacture may include an immunoconjugate of the invention in place of or in addition to an anti-Abeta antibody.

## EXEMPLARY EMBODIMENTS

**[0266]** Provided herein are exemplary embodiments, for illustration.

1. A method of reducing the decline in functional or cognitive capacity in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to a patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in functional or cognitive capacity in the patient.

2. The method of **embodiment 1**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

3. The method of **claim 1**, wherein the antibody is an IgG4 antibody.

4. The method of **embodiment 2 or 3**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

5. The method of **embodiment 4**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

6. The method of **embodiment 5**, wherein the antibody is crenezumab.

7. The method of **any one of the preceding embodiments**, wherein decline in cognitive capacity is assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12), 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

8. The method of **embodiment 7**, wherein the patient is ApoE4 positive.

9. The method of **embodiment 7**, wherein the patient is suffering from mild AD.

10. The method of **embodiment 7**, wherein the patient is suffering from early AD.

11. The method of **any one of embodiments 1 to 8**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

12. The method of **embodiment 11**, wherein the patient has an MMSE between 22 and 26.

13. The method of **any one of the preceding embodiments**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

14. The method of **embodiment 13**, wherein the antibody is administered at a dose of at least 15 mg/kg.

15. The method of **embodiment 14**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

16. The method of **embodiment 13 or 14**, wherein the antibody is administered via intravenous injection.

17. The method of **any one of embodiments 13 to 16**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

18. A method of treating early or mild to moderate AD without increasing the risk of an adverse event comprising administering to a patient diagnosed with early or mild to moderate AD an amount of a humanized monoclonal anti-Aβ antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) that is effective to treat the AD without increasing the risk of a treatment emergent adverse event, wherein the adverse event is selected from: (i) Amyloid-Related Imaging Abnormality—Edema (ARIA-E) and (ii) Amyloid-Related Imaging Abnormality—Hemorrhage (ARIA-H).

19. The method of **embodiment 18**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

20. The method of **embodiment 18**, wherein the antibody is an IgG4 antibody.

21. The method of **embodiment 19**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

22. The method of **embodiment 21**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

23. The method of **embodiment 22**, wherein the antibody is crenezumab.

24. The method of any one of embodiments **18 to 23**, wherein the patient is ApoE4 positive.

25. The method of **any one of embodiments 18 to 23**, wherein the adverse event is ARIA-E.

26. The method of **embodiment 25**, wherein, if a treatment emergent ARIA-E is detected, administration of the antibody is halted and, optionally, treatment for ARIA-E is administered.

27. The method of **embodiment 26**, further comprising resuming administration of said antibody after the ARIA-E is resolved, wherein the antibody is administered at a lower dose than before administration was halted.

28. The method of **embodiment 18**, wherein if one or more new ARIA-Es is detected in the patient during treatment with said antibody, no more antibody is administered, and, optionally, a corticosteroid is administered to the patient.

29. The method of **embodiment 28**, wherein the patient is ApoE4 positive.

30. A method of reducing the decline in functional or cognitive capacity in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to an ApoE4 positive patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in functional or cognitive capacity in the patient.

31. The method of **embodiment 30**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

32. The method of **embodiment 30**, wherein the antibody is an IgG4 antibody.

33. The method of **embodiment 31 or 32**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

34. The method of **embodiment 33**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

35. The method of **embodiment 34**, wherein the antibody is crenezumab.

36. The method of **any one of embodiments 30 to 35**, wherein decline in cognitive capacity capacity is assessed by determining the patient's score before and after administration of said antibody using an ADAS-Cog12, ADAS-Cog13, or ADAS-Cog14 test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

37. The method of **embodiment 36**, wherein the patient has mild AD.

38. The method of **embodiment 36**, wherein the patient has early AD.

39. The method of **any one of embodiments 30 to 37**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

40. The method of **embodiment 39**, wherein the patient has an MMSE score between 22 and 26.

41. The method of **any one of embodiments 30 to 39**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

42. The method of **embodiment 41**, wherein the antibody is administered at a dose of at least 15 mg/kg.

43. The method of **embodiment 42**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

44. The method of **embodiment 41 or 42**, wherein the antibody is administered via intravenous injection.

45. The method of **any one of embodiments 41 to 44**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

46. A method of treating early or mild to moderate AD without increasing the risk of an adverse event comprising administering to an ApoE4 positive patient diagnosed with early or mild to moderate AD an amount of a humanized monoclonal anti-Aβ antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) that is effective to treat the AD without increasing the risk of a treatment emergent adverse event, wherein the adverse event is selected from: (i) Amyloid-Related Imaging Abnormality—Edema (ARIA-E) and (ii) Amyloid-Related Imaging Abnormality—Hemorrhage (ARIA-H).

47. The method of **embodiment 46**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

48. The method of **embodiment 46**, wherein the antibody is an IgG4 antibody.

49. The method of **embodiment 47**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

50. The method of **embodiment 49**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

51. The method of **embodiment 50**, wherein the antibody is crenezumab.

52. The method of **any one of embodiments 46 to 51**, wherein the adverse event is ARIA-E.

53. The method of **embodiment 52**, wherein if a treatment emergent ARIA-E is detected, administration of the antibody is halted and, optionally, treatment for ARIA-E is administered.

54. The method of **embodiment 53**, further comprising resuming administration of said antibody after the ARIA-E is resolved, optionally comprising resuming administration of said antibody at a lower dose than before administration was halted.

55. The method of **embodiment 46**, wherein if one or more new ARIA-Es is detected in the patient during treatment with said antibody, no more antibody is administered, and, optionally, a corticosteroid is administered to the patient.

56. The method of **any one of the preceding embodiments**, wherein the patient is concurrently treated with one or more agents selected from the group consisting of: a therapeutic agent that specifically binds to a target; a cholinesterase inhibitor; an NMDA receptor antagonist; a monoamine depletor; an ergoloid mesylate; an anticholinergic antiparkinsonism agent; a dopaminergic antiparkinsonism agent; a tetrabenazine; an anti-inflammatory agent; a hormone; a vitamin; a dimebolin; a homotaurine; a serotonin receptor activity modulator; an interferon, and a glucocorticoid; an anti-Abeta antibody other than crenezumab; an antibiotic; an anti-viral agent.

57. The method of **embodiment 56**, wherein the agent is a cholinesterase inhibitor.

58. The method of **embodiment 57**, wherein the cholinesterase inhibitor is selected from the group consisting of galantamine, donepezil, rivastigmine and tacrine.

59. The method of **embodiment 56**, wherein the agent is an NMDA receptor antagonist.

60. The method of **embodiment 59**, wherein the NMDA receptor antagonist is memantine or a salt thereof.

61. The method of **embodiment 56**, wherein the agent is a therapeutic agent that specifically binds to a target and the target is selected from the group consisting of beta secretase, tau, presenilin, amyloid precursor protein or portions thereof, amyloid beta peptide or oligomers or fibrils thereof, death receptor 6 (DR6), receptor for advanced glycation endproducts (RAGE), parkin, and huntingtin.

62. The method of **embodiment 56**, wherein the agent is a monoamine depletory, optionally tetrabenazine.

63. The method of **embodiment 56**, wherein the agent is an anticholinergic antiparkinsonism agent selected from the group consisting of procyclidine, diphenhydramine, trihexylphenidyl, benztropine, biperiden and trihexyphenidyl.

64. The method of **embodiment 56**, wherein the agent is a dopaminergic antiparkinsonism agent selected from the group consisting of: entacapone, selegiline, pramipexole, bromocriptine, rotigotine, selegiline, ropinirole, rasagiline, apomorphine, carbidopa, levodopa, pergolide, tolcapone and amantadine.

65. The method of **embodiment 56**, wherein the agent is an anti-inflammatory agent selected from the group consisting of: a nonsteroidal anti-inflammatory drug and indomethacin.

66. The method of **embodiment 56**, wherein the agent is a hormone selected from the group consisting of: estrogen, progesterone and leuprolide.

67. The method of **embodiment 56**, wherein the agent is a vitamin selected from the group consisting of: folate and nicotinamide.

68. The method of **embodiment 56**, wherein the agent is a homotaurine, which is 3-aminopropanesulfonic acid or 3APS.

69. The method of **embodiment 56**, wherein the agent is xaliproden.

70. A method of slowing clinical decline in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to a patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in the patient.

71. The method of **embodiment 70**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

72. The method of **embodiment 70**, wherein the antibody is an IgG4 antibody.

73. The method of **embodiment 71 or 72**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

74. The method of **embodiment 73**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

75. The method of **embodiment 74**, wherein the antibody is crenezumab.

76. The method of **any one of embodiments 70 to 75**, further comprising a decline in cognitive capacity assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12), a 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or a 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

77. The method of **embodiment 76**, wherein the patient is ApoE4 positive.

78. The method of **embodiment 76**, wherein the patient is suffering from mild AD.

79. The method of **embodiment 76**, wherein the patient is suffering from early AD.

80. The method of **any one of embodiments 70 to 78**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

81. The method of **embodiment 80**, wherein the patient has an MMSE score between 22 and 26.

82. The method of **any one of embodiments 70 to 80**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

83. The method of **embodiment 82**, wherein the antibody is administered at a dose of at least 15 mg/kg.

84. The method of **embodiment 83**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

85. The method of **embodiment 82 or 83**, wherein the antibody is administered via intravenous injection.

86. The method of **any one of embodiments 82 to 85**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

87. A method of treating early or mild AD in a subject, comprising administering to a patient suffering from early or mild AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to treat the AD.

88. The method of **embodiment 87**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

89. The method of **embodiment 87**, wherein the antibody is an IgG4 antibody.

90. The method of **embodiment 88 or 89**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

91. The method of **embodiment 90**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

92. The method of **embodiment 91**, wherein the antibody is crenezumab.

93. The method of **any one of embodiments 87 to 92**, wherein the amount is effective to reduce decline in cognitive capacity, which is assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) ), a 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or a 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as

measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

94. The method of **embodiment 93**, wherein the patient is ApoE4 positive.

95. The method of **any one of embodiments 87 to 94**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

96. The method of **embodiment 95**, wherein the patient has an MMSE score between 22 and 26.

97. The method of **any one of embodiments 87 to 95**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

98. The method of **embodiment 97**, wherein the antibody is administered at a dose of at least 15 mg/kg.

99. The method of **embodiment 98**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

100. The method of **embodiment 97 or 98**, wherein the antibody is administered via intravenous injection.

101. The method of **any one of embodiments 97 to 100**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

102. The method of **any one of embodiments 70 to 101**, wherein the patient is concurrently treated with one or more agents selected from the group consisting of: a therapeutic agent that specifically binds to a target; a cholinesterase inhibitor; an NMDA receptor antagonist; a monoamine depletor; an ergoloid mesylate; an anticholinergic antiparkinsonism agent; a dopaminergic antiparkinsonism agent; a tetrabenazine; an anti-inflammatory agent; a hormone; a vitamin; a dimebolin; a homotaurine; a serotonin receptor activity modulator; an interferon, and a glucocorticoid; an anti-Abeta antibody; an antibiotic; an anti-viral agent.

103. The method of **embodiment 102**, wherein the agent is a cholinesterase inhibitor.

104. The method of **embodiment 103**, wherein the cholinesterase inhibitor is selected from the group consisting of galantamine, donepezil, rivastigmine and tacrine.

105. The method of **embodiment 102**, wherein the agent is an NMDA receptor antagonist.

106. The method of **embodiment 105**, wherein the NMDA receptor antagonist is memantine or a salt thereof.

107. The method of **embodiment 102**, wherein the agent is a therapeutic agent that specifically binds to a target and the target is selected from the group consisting of beta secretase, tau, presenilin, amyloid precursor protein or portions thereof, amyloid beta peptide or oligomers or fibrils thereof, death receptor 6 (DR6), receptor for advanced glycation endproducts (RAGE), parkin, and huntingtin.

108. The method of **embodiment 102**, wherein the agent is a monoamine depletory, optionally tetrabenazine.

109. The method of **embodiment 102**, wherein the agent is an anticholinergic antiparkinsonism agent selected from the group consisting of procyclidine, diphenhydramine, trihexylphenidyl, benztropine, biperiden and trihexyphenidyl.

110. The method of **embodiment 102**, wherein the agent is a dopaminergic antiparkinsonism agent selected from the group consisting of: entacapone, selegiline, pramipexole, bromocriptine, rotigotine, selegiline, ropinirole, rasagiline, apomorphine, carbidopa, levodopa, pergolide, tolcapone and amantadine.

111. The method of **embodiment 102**, wherein the agent is an anti-inflammatory agent selected from the group consisting of: a nonsteroidal anti-inflammatory drug and indomethacin.

112. The method of **embodiment 102**, wherein the agent is a hormone selected from the group consisting of: estrogen, progesterone and leuprolide.

113. The method of **embodiment 102**, wherein the agent is a vitamin selected from the group consisting of: folate and nicotinamide.

114. The method of **embodiment 102**, wherein the agent is a homotaurine, which is 3-aminopropanesulfonic acid or 3APS.

115. The method of **embodiment 102**, wherein the agent is xaliproden.

116. The method of of **embodiment 102**, wherein the agent is an anti-Abeta antibody other than crenezumab.

## EXAMPLES

### EXAMPLE 1 -- Clinical Study of Crenezumab, a Humanized Anti-Aβ Monoclonal Antibody, in the Treatment of Mild to Moderate Alzheimer's Disease

**Study Design and Objectives**

**[0267]** A randomized, double blind Phase II trial was conducted, using a placebo control, to evaluate the impact of the humanized monoclonal anti-amyloid beta ("Aβ") antibody crenezumab in patients diagnosed with mild to moderate Alzheimer's Disease (AD). Patients included in the study were, at the time of screening, between the ages of 50 and 80, and had a diagnosis of probable AD according to the NINCDS-ADRDA criteria with: a Mini-Mental State Examination (MMSE) score of 18 to 26 points, a Geriatric Depression Scale (GDS-15) score of less than 6, completion of 6 years of education (or good work history consistent with exclusion of mental retardation or other pervasive developmental disorders). Additionally, for those patients receiving concurrent AD treatment (such as acetylcholinesterase inhibitors or memantine), the patient was confirmed to have been on the medication for at least 3 months and at a stable dose for at least 2 months prior to randomization. At least 50% of the enrolled patients were ApoE4 positive (carrying at least one ApoE4 allele). Patients concurrently receiving one or more non-excluded prescription or over the counter medication, such as non-anticholinergic antidepressant(s), atypical antipsychotic(s), non-benzodiazepine anxiolytic(s), soporific(s), centrally acting anticholinergic antihistamine(s), and centrally acting anticholinergic antispasmodic(s), were also allowed to enroll provided that the dose administered was constant for at least 1 month prior to randomization and remained the same for the duration of the study.

**[0268]** Individuals were excluded from the trial if: they suffer from a severe or unstable medical condition that, in the opinion of the investigator or sponsor, would interfere with the patient's ability to complete the study assessments or would require the equivalent of institutional or hospital care; there is a history or presence of clinically evident vascular disease potentially affecting the brain; there is a history of severe, clinically significant central nervous system trauma (such as persistent neurological deficit or structural brain damage); they have been hospitalized in the 4 weeks before screening; they have previously been treated with crenezumab or any other agent that targets Aβ; or if they have received treatment with any biological therapy (other than routine vaccinations) within the longer of 5 half-lives

of the therapeutic agent in the biological therapy or 3 months before screening.

**[0269]** The study had three periods – a screening period lasting up to 35 days, a treatment period lasting 68 weeks (referred to herein as Week 1, Week 2, etc., up to Week 69), and a safety follow-up period lasting a further 16 weeks (referred to herein as Week 70, etc., up to Week 85). Treatment (or placebo) was administered via intravenous infusion.

**[0270]** Patients are enrolled in the trial and randomized into one of two arms, a treatment (*i.e.*, crenezumab) arm and a placebo arm in a 2:1 (treatment arm:placebo arm) randomization. 249 patients with an MMSE score from 18 to 26 (categorized as mild to moderate AD) were enrolled in the trial, of whom 165 received treatment and 84 received placebo. 121 patients in treatment arm and 61 patients in the placebo arm had an MMSE score between 20 and 26 (categorized as mild AD). Within the treatment art 117 (or 70.9%) were ApoE4 positive. In the placebo arm, 60 patients (or 71.4%) were ApoE4 positive. *See* **FIGS. 4A-B** (Tabulating Patient Disposition).

**[0271]** A safety run-in assessment of 43 days was performed to determine the safety and tolerability of a 15 mg/kg intravenous dose versus a 10 mg/kg intravenous dose and a dose of 15 mg/kg was chosen. Patients in both arms of the trial received a blinded intravenous injection every four weeks for 68 weeks; based on the results of the safety run-in, patients in the treatment arm receive 15 mg/kg, while patients in the placebo arm received an intravenous injection of placebo. *See* **FIG.5** (Protocol Schematic).

**[0272]** Patients were assessed after 72 weeks for (a) change in ADAS-Cog12 score and CDR-SOB score at Weeks 25, 49, and 73 from the baseline score at the start of trial, to evaluate inhibition of disease progression and (b) safety and tolerability of crenezumab as compared to placebo. To estimate statistical significance of any measured change, analysis of covariance, confidence intervals, and least squares estimates of the difference in the mean change from baseline were calculated.

**[0273]** The safety and tolerability of crenezumab was assessed by measuring the frequency and severity of treatment emergent adverse events throughout the trial, especially instances of symptomatic or asymptomatic ARIA-E (including cerebral vasogenic edema), symptomatic or asymptomatic ARIA-H (including cerebral microhemorrhage), and cerebral macrohemorrhage. The presence and/or number of cerebral vasogenic edema cases during the screening period (before the start of dosing) or during the treatment period (after the start of dosing with placebo or crenezumab) was assessed by fluid attenuated inversion recovery magnetic resonance imaging (FLAIR MRI). *See, e.g.*, Sperling *et al.*, 2011, Alzheimer's &

Dementia 7:367-385. The presence and/or number of cerebral microhemorrhage(s) during the screening period (before the start of dosing) or during the treatment period (after the start of dosing with placebo or crenezumab) was assessed by transverse magnetization relaxation time with additional inhomogeneous dephasing gradient recalled echo magnetic resonance imaging (T2*-weighted GRE MRI).

**Results**

**[0274]** ADAS-Cog12 measurements at 73 weeks demonstrate that patients received crenezumab showed less disease progression than patients who received placebo. As summarized in the tables shown in **FIG. 6A-B** and in the charts shown in **FIGS. 7-8**, the change in the ADAS-Cog12 score was about 24% (p=0.12) less in the treatment arm than in the placebo arm for patients with mild AD and about 16% (p=0.19) less in the treatment arm than in the placebo arm, for patients with mild to moderate AD. This effect was also seen between ApoE4 positive patients in the treatment arm versus placebo arm: there was 24.4% (p=0.08, not adjusted for multiplicity) less increase in the ADAS-Cog12 score (where an increase in the ADAS-Cog12 score is indicative of disease progression) in the patients receiving crenezumab relative to the patients receiving placebo. *See* **FIG. 6A** and **FIG. 9**. The ApoE4 positive patients included patients with both mild and moderate AD. The effect was even more pronounced when the results for both mild and ApoE4 positive patients were pooled: a reduction of 32.4% (p=0.05, not adjusted for multiplicity) was seen in the treatment arm relative to the placebo arm. *See* **FIG. 6A** and **FIG. 10**. The treatment effect increased with increasing MMSE score at enrollment. As shown in FIG. 6B, the higher the MMSE score, the greater the percent reduction in ADAS-Cog12 in the treatment arm versus the placebo arm, ranging from about 16% for patients with an MMSE between 18-26 up to a 49% reduction in patients with an MMSE between 25 and 26. *See also*, **FIG 11**. For patients, having an MMSE score between 22 and 26, the percent reduction in ADAS-Cog12 in the treatment arm compared to the placebo arm was about 35%.

**[0275]** The change in CDR-SOB showed a similar trend in treatment effect. As shown in **FIG. 12A**, a 19% reduction in the change in CDR-SOB scores was seen in the treatment arm versus placebo for patients having an MMSE of 22-26, and this effect was even more pronounced in patients having an MMSE score of 25-26, where the percent reduction was about 63% (see also **FIG 13**). **FIG. 12B** shows that when looking at the Memory or the Judgment and Problem solving component scores for patients having an MMSE of 22-26, the percent reduction was about 42% and 30% respectively.

**[0276]** The study further demonstrated that crenezumab did not increase the incidence of ARIA-type events when dosed at 15 mg/kg. A single, asymptomatic ARIA-E event was observed in the study, in a patient receiving crenezumab. The number of ARIA-H incidents was balanced between the treatment and placebo arms.

**[0277]** These data demonstrate that crenezumab inhibits disease progression without increasing the incidence of a treatment emergent adverse event such as ARIA-E or ARIA-H when administered at a dose of 15 mg/kg in patients suffering from mild to moderate AD, particularly in patients with mild AD and/or who are ApoE4 positive.

## EXAMPLE 2 -- Clinical Study of Crenezumab, a Humanized Anti-Aβ Monoclonal Antibody, in the Treatment of Mild to Moderate Alzheimer's Disease and to Evaluate the Impact on Amyloid Load

### Study Design and Objectives

**[0278]** A randomized, double blind Phase II trial was conducted, using a placebo control, to evaluate the impact of the humanized monoclonal anti-amyloid beta ("Aβ") antibody crenezumab in patients diagnosed with mild to moderate Alzheimer's Disease (AD). Patients included in the study were, at the time of screening, between the ages of 50 and 80, and had a diagnosis of probable AD according to the NINCDS-ADRDA criteria with: a Mini-Mental State Examination (MMSE) score of 18 to 26 points, a Geriatric Depression Scale (GDS-15) score of less than 6, completion of 6 years of education (or good work history consistent with exclusion of mental retardation or other pervasive developmental disorders). Only patients with a positive florbetapir PET ("amyloid positive") scan at screening, indicative of increased brain amyloid load in the range expected for patients diagnosed with AD as assessed by florbetapir-PET scan, were enrolled. Additionally, at least 50% of the enrolled patients were ApoE4 positive.

**[0279]** Patients were enrolled in the trial and randomized into one of two arms, a treatment (*i.e.*, crenezumab) arm and a placebo arm in a 2:1 (treatment arm:placebo arm) randomization. 52 patients across both arms of the trial received a blinded intravenous injection every four weeks for 73 weeks. In the treatment arm, patients received a 15 mg/kg dose of crenezumab. Patients were stratified according to: ApoE4 status (carrier versus non-carrier) and MMSE score.

**[0280]** Data were collected for changes in: ADAS-Cog12, amyloid burden as measured using florbetapir-PET, and Abeta levels in cerebrospinal fluid (CSF). Florbetapir PET scans were acquired at the screening, 12 month, and 18 month visits using florbetapir 10 mCi, with

a 50-min. uptake period and 30 min. emission scan. Images from 6X5 minute frames (or 1X15 minute frames on scanners without dynamic capability) were normalized to standard space where a template was used to extract the mean signals from several regions of interest (ROIs). Baseline T1-weighted MRI scans were used to refine the volumes of the template ROIs. Analyses were conducted using cerebellar cortex or subcortical white matter as a reference region. CSF was collected at screening and prior to dosing at month 18. CSF Abeta, tau and p-tau(181) were measured. ANCOVA or mixed model for repeated measures was used for statistical analysis of treatment differences at study endpoints.

**[0281]** Patient characteristics, adverse events, and timing of PET scans, MRI scans, and CSF sampling are shown in **FIG. 14A-B**.

**[0282]** **Results.** ADAS-Cog12 measurements at the end of the treatment period demonstrate that patients who received crenezumab showed less disease progression than patients who received placebo. A 54.3% reduction in cognitive decline was observed in patients with an initial MMSE score between 20 and 26 (p=0.2). Consistent with this observed slowing in disease progression, a decrease in the accumulation of amyloid deposits was also observed by PET analysis (with a subcortical white matter reference region) in patients treated with crenezumab versus patient receiving placebo. *See* **FIG. 15A**. Furthermore, an increase in cerebrospinal fluid concentration of Abeta was detected in the treatment arm, consistent with engagement of the target by crenezumab. *See* **FIG. 15B**. A similar increase in cerebrospinal fluid concentration of Abeta was detected in patients treated with 300 mg subcutaneous administration of crenezumab every two weeks versus patients receiving placebo.

**[0283]** These data demonstrate that crenezumab engages its target, amyloid beta, and inhibits disease progression when administered at a dose of 15 mg/kg in patients suffering from mild to moderate AD, particularly in patients with mild AD, including in patients having a brain amyloid burden that is typical of that seen in patients diagnosed with AD.

**[0284]** Although the foregoing invention has been described in some detail by way of illustration and example for purposes of clarity of understanding, the descriptions and examples should not be construed as limiting the scope of the invention. The disclosures of all patent applications and publications and scientific literature cited herein are expressly incorporated in their entirety by reference for any purpose.

**[0285]** SEQUENCE LISTING KEY

| SEQ ID NO: | Sequence |
|---|---|
| 1 | Human Aβ1-42 amino acid sequence: DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA |
| 2 | Crenezumab HVR-H1 amino acid sequence: GFTFSSYGMS |
| 3 | Crenezumab HVR-H2 amino acid sequence: SINSNGGSTYYPDSVK |
| 4 | Crenezumab HVR-H3 amino acid sequence: GDY |
| 5 | Crenezumab heavy chain amino acid sequence (HVR regions marked in bold text): EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSSYGMS**WVRQAPGK GLELVA**SINSNGGSTYYPDSVK**GRFTISRDNAKNSLYLQMNSLR AEDTAVYYCAS**GDY**WGQGTTVTVSSASTKGPSVFPLAPCSRSTS ESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS LSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPC PAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLG |
| 6 | Crenezumab HVR-L1 amino acid sequence: RSSQSLVYSNGDTYLH |
| 7 | Crenezumab HVR-L2 amino acid sequence: KVSNRFS |
| 8 | Crenezumab HVR-L3 amino acid sequence: SQSTHVPWT |
| 9 | Crenezumab light chain amino acid sequence (HVR regions marked in bold and underlined text): DIVMTQSPLSLPVTPGEPASISC**RSSQSLVYSNGDTYLH**WYLQKP GQSPQLLIY**KVSNRFS**GVPDRFSGSGSGTDFTLKISRVEAEDVGV YYC**SQSTHVPWT**FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC |

**CLAIMS:**

1. A method of reducing the decline in functional or cognitive capacity in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to a patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in functional or cognitive capacity in the patient.

2. The method of **claim 1**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

3. The method of **claim 1**, wherein the antibody is an IgG4 antibody.

4. The method of **claim 2 or 3**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

5. The method of **claim 4**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

6. The method of **claim 5**, wherein the antibody is crenezumab.

7. The method of **any one of the preceding claims**, wherein decline in cognitive capacity is assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12), 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

8. The method of **claim 7**, wherein the patient is ApoE4 positive.

9. The method of **claim 7**, wherein the patient is suffering from mild AD.

10. The method of **claim 7**, wherein the patient is suffering from early AD.

11. The method of **any one of claims 1 to 8**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

12. The method of **claim 11**, wherein the patient has an MMSE between 22 and 26.

13. The method of **any one of the preceding claims**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

14. The method of **claim 13**, wherein the antibody is administered at a dose of at least 15 mg/kg.

15. The method of **claim 14**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

16. The method of **claim 13 or 14**, wherein the antibody is administered via intravenous injection.

17. The method of **any one of claims 13 to 16**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

18. A method of treating early or mild to moderate AD without increasing the risk of an adverse event comprising administering to a patient diagnosed with early or mild to moderate AD an amount of a humanized monoclonal anti-Aβ antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) that is effective to treat the AD without increasing the risk of a treatment emergent adverse event, wherein the adverse event is selected from: (i) Amyloid-Related Imaging Abnormality—Edema (ARIA-E) and (ii) Amyloid-Related Imaging Abnormality—Hemorrhage (ARIA-H).

19. The method of **claim 18**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

20. The method of **claim 18**, wherein the antibody is an IgG4 antibody.

21. The method of **claim 19**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

22. The method of **claim 21**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

23. The method of **claim 22**, wherein the antibody is crenezumab.

24. The method of any one of claims **18 to 23**, wherein the patient is ApoE4 positive.

25. The method of **any one of claims 18 to 23**, wherein the adverse event is ARIA-E.

26. The method of **claim 25**, wherein if a treatment emergent ARIA-E is detected, administration of the antibody is halted and, optionally, treatment for ARIA-E is administered.

27. The method of **claim 26**, further comprising resuming administration of said antibody after the ARIA-E is resolved, wherein the antibody is administered at a lower dose than before administration was halted.

28. The method of **claim 18**, wherein if one or more new ARIA-Es is detected in the patient during treatment with said antibody, no more antibody is administered, and, optionally, a corticosteroid is administered to the patient.

29. The method of **claim 28**, wherein the patient is ApoE4 positive.

30. A method of reducing the decline in functional or cognitive capacity in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to an ApoE4 positive patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24

of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in functional or cognitive capacity in the patient.

31. The method of **claim 30**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

32. The method of **claim 30**, wherein the antibody is an IgG4 antibody.

33. The method of **claim 31 or 32**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

34. The method of **claim 33**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

35. The method of **claim 34**, wherein the antibody is crenezumab.

36. The method of **any one of claims 30 to 35**, wherein decline in cognitive capacity is assessed by determining the patient's score before and after administration of said antibody using an ADAS-Cog12, ADAS-Cog13, or ADAS-Cog14 test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

37. The method of **claim 36**, wherein the patient has mild AD.

38. The method of **claim 36**, wherein the patient has early AD.

39. The method of **any one of claims 30 to 37**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

40. The method of **claim 39**, wherein the patient has an MMSE score between 22 and 26.

41. The method of **any one of claims 30 to 39**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

42. The method of **claim 41**, wherein the antibody is administered at a dose of at least 15 mg/kg.

43. The method of **claim 42**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

44. The method of **claim 41 or 42**, wherein the antibody is administered via intravenous injection.

45. The method of **any one of claims 41 to 44**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

46. A method of treating early or mild to moderate AD without increasing the risk of an adverse event comprising administering to an ApoE4 positive patient diagnosed with early or mild to moderate AD an amount of a humanized monoclonal anti-Aβ antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) that is effective to treat the AD without increasing the risk of a treatment emergent adverse event, wherein the adverse event is selected from: (i) Amyloid-Related Imaging Abnormality—Edema (ARIA-E) and (ii) Amyloid-Related Imaging Abnormality—Hemorrhage (ARIA-H).

47. The method of **claim 46**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

48. The method of **claim 46**, wherein the antibody is an IgG4 antibody.

49. The method of **claim 47**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

50. The method of **claim 49**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

51. The method of **claim 50**, wherein the antibody is crenezumab.

52. The method of **any one of claims 46 to 51**, wherein the adverse event is ARIA-E.

53. The method of **claim 52**, wherein if a treatment emergent ARIA-E is detected, administration of the antibody is halted and, optionally, treatment for ARIA-E is administered.

54. The method of **claim 53**, further comprising resuming administration of said antibody after the ARIA-E is resolved, optionally comprising resuming administration of said antibody at a lower dose than before administration was halted.

55. The method of **claim 46**, wherein if one or more new ARIA-Es is detected in the patient during treatment with said antibody, no more antibody is administered, and, optionally, a corticosteroid is administered to the patient.

56. The method of **any one of the preceding claims**, wherein the patient is concurrently treated with one or more agents selected from the group consisting of: a therapeutic agent that specifically binds to a target; a cholinesterase inhibitor; an NMDA receptor antagonist; a monoamine depletor; an ergoloid mesylate; an anticholinergic antiparkinsonism agent; a dopaminergic antiparkinsonism agent; a tetrabenazine; an anti-inflammatory agent; a hormone; a vitamin; a dimebolin; a homotaurine; a serotonin receptor activity modulator; an interferon, and a glucocorticoid; an anti-Abeta antibody other than crenezumab; an antibiotic; an anti-viral agent.

57. The method of **claim 56**, wherein the agent is a cholinesterase inhibitor.

58. The method of **claim 57**, wherein the cholinesterase inhibitor is selected from the group consisting of galantamine, donepezil, rivastigmine and tacrine.

59. The method of **claim 56**, wherein the agent is an NMDA receptor antagonist.

60. The method of **claim 59**, wherein the NMDA receptor antagonist is memantine or a salt thereof.

61. The method of **claim 56**, wherein the agent is a therapeutic agent that specifically binds to a target and the target is selected from the group consisting of beta secretase, tau, presenilin, amyloid precursor protein or portions thereof, amyloid beta peptide or oligomers or fibrils thereof, death receptor 6 (DR6), receptor for advanced glycation endproducts (RAGE), parkin, and huntingtin.

62. The method of **claim 56**, wherein the agent is a monoamine depletory, optionally tetrabenazine.

63. The method of **claim 56**, wherein the agent is an anticholinergic antiparkinsonism agent selected from the group consisting of procyclidine, diphenhydramine, trihexylphenidyl, benztropine, biperiden and trihexyphenidyl.

64. The method of **claim 56**, wherein the agent is a dopaminergic antiparkinsonism agent selected from the group consisting of: entacapone, selegiline, pramipexole, bromocriptine, rotigotine, selegiline, ropinirole, rasagiline, apomorphine, carbidopa, levodopa, pergolide, tolcapone and amantadine.

65. The method of **claim 56**, wherein the agent is an anti-inflammatory agent selected from the group consisting of: a nonsteroidal anti-inflammatory drug and indomethacin.

66. The method of **claim 56**, wherein the agent is a hormone selected from the group consisting of: estrogen, progesterone and leuprolide.

67. The method of **claim 56**, wherein the agent is a vitamin selected from the group consisting of: folate and nicotinamide.

68. The method of **claim 56**, wherein the agent is a homotaurine, which is 3-aminopropanesulfonic acid or 3APS.

69. The method of **claim 56**, wherein the agent is xaliproden.

70. A method of slowing clinical decline in a patient diagnosed with early or mild to moderate Alzheimer's Disease (AD) comprising administering to a patient suffering from early or mild to moderate AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to slow the decline in the patient.

71. The method of **claim 70**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

72. The method of **claim 70**, wherein the antibody is an IgG4 antibody.

73. The method of **claim 71 or 72**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

74. The method of **claim 73**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

75. The method of **claim 74**, wherein the antibody is crenezumab.

76. The method of **any one of claims 70 to 75**, further comprising slowing a decline in cognitive capacity, wherein the decline in cognitive capacity is assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12), a 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or a 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

77. The method of **claim 76**, wherein the patient is ApoE4 positive.

78. The method of **claim 76**, wherein the patient is suffering from mild AD.

79. The method of **claim 76**, wherein the patient is suffering from early AD.

80. The method of **any one of claims 70 to 78**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

81. The method of **claim 80**, wherein the patient has an MMSE score between 22 and 26.

82. The method of **any one of claims 70 to 80**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

83. The method of **claim 82**, wherein the antibody is administered at a dose of at least 15 mg/kg.

84. The method of **claim 83**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

85. The method of **claim 82 or 83**, wherein the antibody is administered via intravenous injection.

86. The method of **any one of claims 82 to 85**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

87. A method of treating early or mild AD in a subject, comprising administering to a patient suffering from early or mild AD a humanized monoclonal anti-amyloid beta (Aβ) antibody that binds within residues 13 and 24 of amyloid β (1-42)(SEQ ID NO:1) in an amount effective to treat the AD.

88. The method of **claim 87**, wherein the antibody is capable of binding oligomeric and monomeric forms of amyloid β.

89. The method of **claim 87**, wherein the antibody is an IgG4 antibody.

90. The method of **claim 88 or 89**, wherein the antibody comprises six hypervariable regions (HVRs), wherein:

(i) HVR-H1 is SEQ ID NO:2;

(ii) HVR-H2 is SEQ ID NO:3;

(iii) HVR-H3 is SEQ ID NO:4;

(iv) HVR-L1 is SEQ ID NO:6;

(v) HVR-L2 is SEQ ID NO:7; and

(vi) HVR-L3 is SEQ ID NO:8.

91. The method of **claim 90**, wherein the antibody comprises a heavy chain having the amino acid sequence of SEQ ID NO:5 and a light chain having the amino acid sequence of SEQ ID NO:9.

92. The method of **claim 91**, wherein the antibody is crenezumab.

93. The method of **any one of claims 87 to 92**, wherein the amount is effective to reduce decline in cognitive capacity assessed by determining the patient's score before and after administration of said antibody using a 12-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) ), a 13-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog13), or a 14-item Alzheimer's Disease Assessment Scale – Cognition (ADAS-Cog12) test, optionally wherein the reduction in cognitive decline as measured by ADAS-Cog is at least 30%, at least 35%, at least 40%, or at least 45% relative to placebo.

94. The method of **claim 93**, wherein the patient is ApoE4 positive.

95. The method of **any one of claims 87 to 94**, wherein the patient has an MMSE score of at least 20, between 20 and 30, between 20 and 26, between 24 and 30, between 21 and 26, between 22 and 26, between 22 and 28, between 23 and 26, between 24 and 26, or between 25 and 26 before initiation of treatment.

96. The method of **claim 95**, wherein the patient has an MMSE score between 22 and 26.

97. The method of **any one of claims 87 to 95**, wherein the antibody is administered at a dose of 10 mg/kg to 100 mg/kg of patient body weight.

98. The method of **claim 97**, wherein the antibody is administered at a dose of at least 15 mg/kg.

99. The method of **claim 98**, wherein the antibody is administered at a dose of 15 mg/kg, 30 mg/kg, 45 mg/kg, 50 mg/kg, or 60 mg/kg.

100. The method of **claim 97 or 98**, wherein the antibody is administered via intravenous injection.

101. The method of **any one of claims 97 to 100**, wherein the antibody is administered every 2 weeks, every 4 weeks, every month, every two months, or every six months.

102. The method of **any one of claims 70 to 101**, wherein the patient is concurrently treated with one or more agents selected from the group consisting of: a therapeutic agent that

specifically binds to a target; a cholinesterase inhibitor; an NMDA receptor antagonist; a monoamine depletor; an ergoloid mesylate; an anticholinergic antiparkinsonism agent; a dopaminergic antiparkinsonism agent; a tetrabenazine; an anti-inflammatory agent; a hormone; a vitamin; a dimebolin; a homotaurine; a serotonin receptor activity modulator; an interferon, and a glucocorticoid; an anti-Abeta antibody; an antibiotic; an anti-viral agent.

103. The method of **claim 102**, wherein the agent is a cholinesterase inhibitor.

104. The method of **claim 103**, wherein the cholinesterase inhibitor is selected from the group consisting of galantamine, donepezil, rivastigmine and tacrine.

105. The method of **claim 102**, wherein the agent is an NMDA receptor antagonist.

106. The method of **claim 105**, wherein the NMDA receptor antagonist is memantine or a salt thereof.

107. The method of **claim 102**, wherein the agent is a therapeutic agent that specifically binds to a target and the target is selected from the group consisting of beta secretase, tau, presenilin, amyloid precursor protein or portions thereof, amyloid beta peptide or oligomers or fibrils thereof, death receptor 6 (DR6), receptor for advanced glycation endproducts (RAGE), parkin, and huntingtin.

108. The method of **claim 102**, wherein the agent is a monoamine depletory, optionally tetrabenazine.

109. The method of **claim 102**, wherein the agent is an anticholinergic antiparkinsonism agent selected from the group consisting of procyclidine, diphenhydramine, trihexylphenidyl, benztropine, biperiden and trihexyphenidyl.

110. The method of **claim 102**, wherein the agent is a dopaminergic antiparkinsonism agent selected from the group consisting of: entacapone, selegiline, pramipexole, bromocriptine, rotigotine, selegiline, ropinirole, rasagiline, apomorphine, carbidopa, levodopa, pergolide, tolcapone and amantadine.

111. The method of **claim 102**, wherein the agent is an anti-inflammatory agent selected from the group consisting of: a nonsteroidal anti-inflammatory drug and indomethacin.

112. The method of **claim 102**, wherein the agent is a hormone selected from the group consisting of: estrogen, progesterone and leuprolide.

113. The method of **claim 102**, wherein the agent is a vitamin selected from the group consisting of: folate and nicotinamide.

114. The method of **claim 102**, wherein the agent is a homotaurine, which is 3-aminopropanesulfonic acid or 3APS.

115. The method of **claim 102**, wherein the agent is xaliproden.

116. The method of of **claim 102**, wherein the agent is an anti-Abeta antibody other than crenezumab.

SEQ ID NO:1

1 DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA

FIG. 1

HVR-H1 (SEQ ID NO:2): GFTFSSYGMS
HVR-H2 (SEQ ID NO:3): SINSNGGSTYYPDSVK
HVR-H3 (SEQ ID NO:4): GDY

HVR-L1 (SEQ ID NO:6): RSSQSLVYSNGDTYLH
HVR-L2 (SEQ ID NO:7): KVSNRFS
HVR-L3 (SEQ ID NO:8): SQSTHVPWT

FIG. 2

HC Sequence (SEQ ID NO:5)

```
  1 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS
 51 INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD
101 YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT
151 VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK
201 PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE
251 VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV
301 LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM
351 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS
401 RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
```

LC Sequence (SEQ ID NO:9)

```
  1 DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ
 51 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP
101 WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
151 VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
201 VTHQGLSSPV TKSFNRGEC
```

FIG. 3

| | IV (N=249) | |
|---|---|---|
| | Placebo (N=84) | Crenezumab (N=165) |
| Status of AD-Conmed Use | | |
| n | 84 | 165 |
| None | 11 (13.1%) | 19 (11.5%) |
| AchEIs Only | 50 (59.5%) | 96 (58.2%) |
| Memantine Only | 2 (2.4%) | 8 (4.8%) |
| AchEIs and Memantine | 21 (25.0%) | 42 (25.5%) |
| ADAS-Cog12: Mild-to-Moderate | | |
| n | 84 | 163 |
| Mean (SD) | 27.08 (7.52) | 28.87 (9.17%) |
| Median | 26.50 | 28.33 |
| Min - Max | 12.7 - 52.0 | 7.3 - 55.0 |

FIG. 4A

| | IV (N=249) | |
|---|---|---|
| | Placebo (N=84) | Crenezumab (N=165) |
| Actual Apoe4 Result | | |
| n | 84 | 165 |
| E2/E3 | 2 (2.4%) | 6 (3.6%) |
| E2/E4 | 4 (4.8%) | 6 (3.6%) |
| E3/E3 | 22 (26.2%) | 42 (25.5%) |
| E3/E4 | 39 (46.4%) | 76 (46.1%) |
| E4/E4 | 17 (20.2%) | 35 (21.2%) |
| APOE4 Carriers | | |
| n | 84 | 165 |
| NEGATIVE | 24 (28.6%) | 48 (29.1%) |
| POSITIVE | 60 (71.4%) | 117 (70.9%) |
| MMSE Distribution | | |
| n | 84 | 165 |
| MILD (20-26) | 61 (72.6%) | 121 (73.3%) |
| MODERATE (18-19) | 23 (27.4%) | 44 (26.7%) |
| MMSE Score at Screening | | |
| n | 84 | 165 |
| Mean (50) | 21.60 (2.51%) | 21.85 (2.72%) |
| Median | 22.00 | 22.00 |
| Min - Max | 18.0 - 26.0 | 16.0 - 26.0 |

FIG. 4B

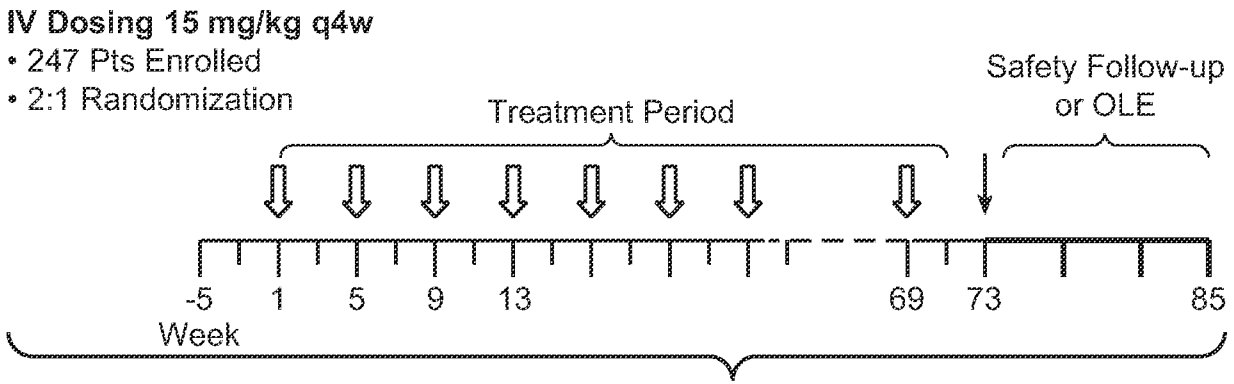


FIG. 5

**IV Cohort ADAS-Cog12: Summary of Week 73 MMRM Results on Observed Case Data**

| Population | N at Week 73 | | Placebo LS Mean (SE) Change Score | Crenezumab LS Mean (SE) Change Score | Difference | | | % Reduction |
|---|---|---|---|---|---|---|---|---|
| | Placebo | Crenezumab | | | LS Mean (SE) | 80% CI | P-value | |
| M2M | 64 | 122 | 10.85 (1.10) | 9.07 (0.81) | 1.78 (1.35) | 0.04, 3.51 | 0.19 | 16% |
| ApoE4 Negative | 21 | 32 | 9.34 (2.04) | 9.83 (1.59) | -0.50 (2.59) | -3.85, 2.86 | 0.849* | -5% |
| ApoE4 Positive | 43 | 90 | 11.19 (1.29) | 8.46 (0.91) | 2.73 (1.58) | 0.70, 4.77 | 0.086* | 24% |
| Mild | 47 | 93 | 10.09 (1.22) | 7.85 (0.90) | 2.24 (1.47) | 0.36, 4.13 | 0.128* | 22% |
| Mild and ApoE4 Positive | 31 | 68 | 9.89 (1.37) | 6.68 (0.97) | 3.21 (1.64) | 1.08, 5.33 | 0.054* | 32% |
| Moderate | 17 | 29 | 13.83 (2.38) | 13.68 (1.89) | 0.16 (2.99) | -3.74, 4.05 | 0.959* | 1% |

* p values not adjusted for multiplicity

FIG. 6A

| IV Cohort ADAS-Cog12: Summary of Week 73 MMRM Results on Observed Case Data | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MMSE Range (% of MITT) | N at Week 73 | | Placebo LS Mean (SE) Change Score | Crenezumab LS Mean (SE) Change Score | Difference | | | % Reduction | ES (SD) |
| | Placebo | Crenezumab | | | LS Mean (SE) | 80% CI | P-value | | |
| 18-26 (100%) | 64 | 122 | 10.56 (1.09) | 8.79 (0.79) | 1.78 (1.35) | 0.04, 3.51 | 0.190 | 16.8% | 0.20 (9.08) |
| 19-26 (87%) | 56 | 105 | 10.18 (1.15) | 8.07 (0.84) | 2.12 (1.42) | 0.29, 3.95 | 0.139 | 20.80% | 0.24 (8.89) |
| 20-26 (74%) | 47 | 93 | 9.43 (1.20) | 7.18 (0.85) | 2.24 (1.47) | 0.36, 4.13 | 0.128 | 23.8% | 0.27 (8.44) |
| 21-26 (64%) | 39 | 83 | 9.22 (1.30) | 6.96 (0.90) | 2.26 (1.58) | 0.22, 4.30 | 0.157 | 24.5% | 0.27 (8.40) |
| 22-26 (54%) | 33 | 70 | 9.70 (1.33) | 6.26 (0.91) | 3.44 (1.61) | 1.36, 5.52 | 0.036 | 35.4% | 0.44 (7.80) |
| 23-26 (43%) | 24 | 60 | 7.92 (1.44) | 5.51 (0.91) | 2.40 (1.70) | 0.20, 4.60 | 0.163 | 30.30% | 0.33 (7.18) |
| 24-26 (32%) | 16 | 45 | 7.41 (1.77) | 4.58 (1.06) | 2.83 (2.07) | 0.15, 5.51 | 0.176 | 38.20% | 0.39 (7.25) |
| 25-26 (21%) | 11 | 30 | 6.88 (2.13) | 3.51 (1.31) | 3.37 (2.50) | 0.11, 6.63 | 0.185 | 49.00% | 0.47 (7.21) |

*FIG. 6B*

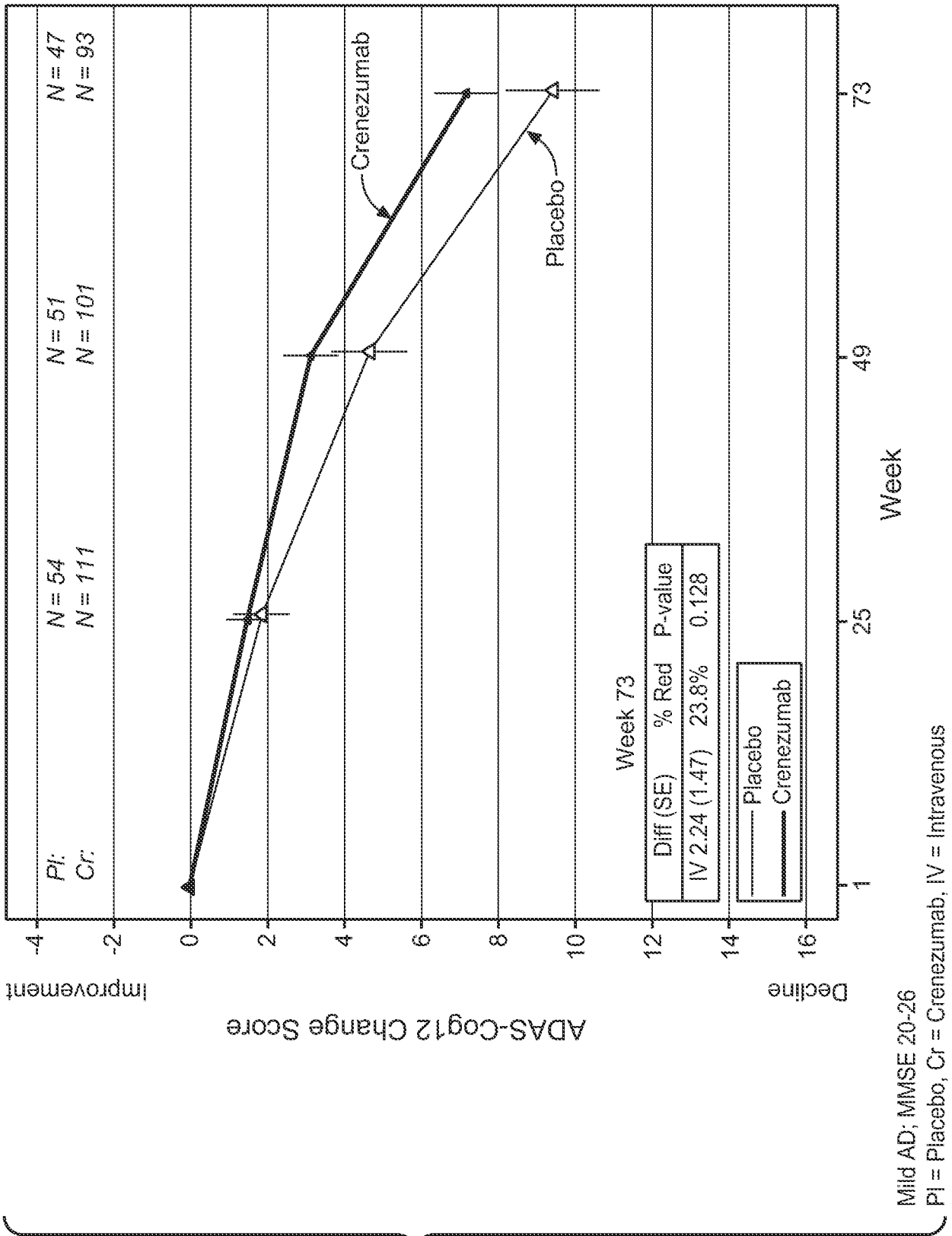
ADAS-Cog12 Change Score
improvement
Decline
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
Week
Placebo
Crenezumab
Pl: N = 54 N = 51 N = 47
Cr: N = 111 N = 101 N = 93
Week 73
Diff (SE) % Red P-value
IV 2.24 (1.47) 23.8% 0.128
Placebo
Crenezumab
Mild AD; MMSE 20-26
Pl = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 7

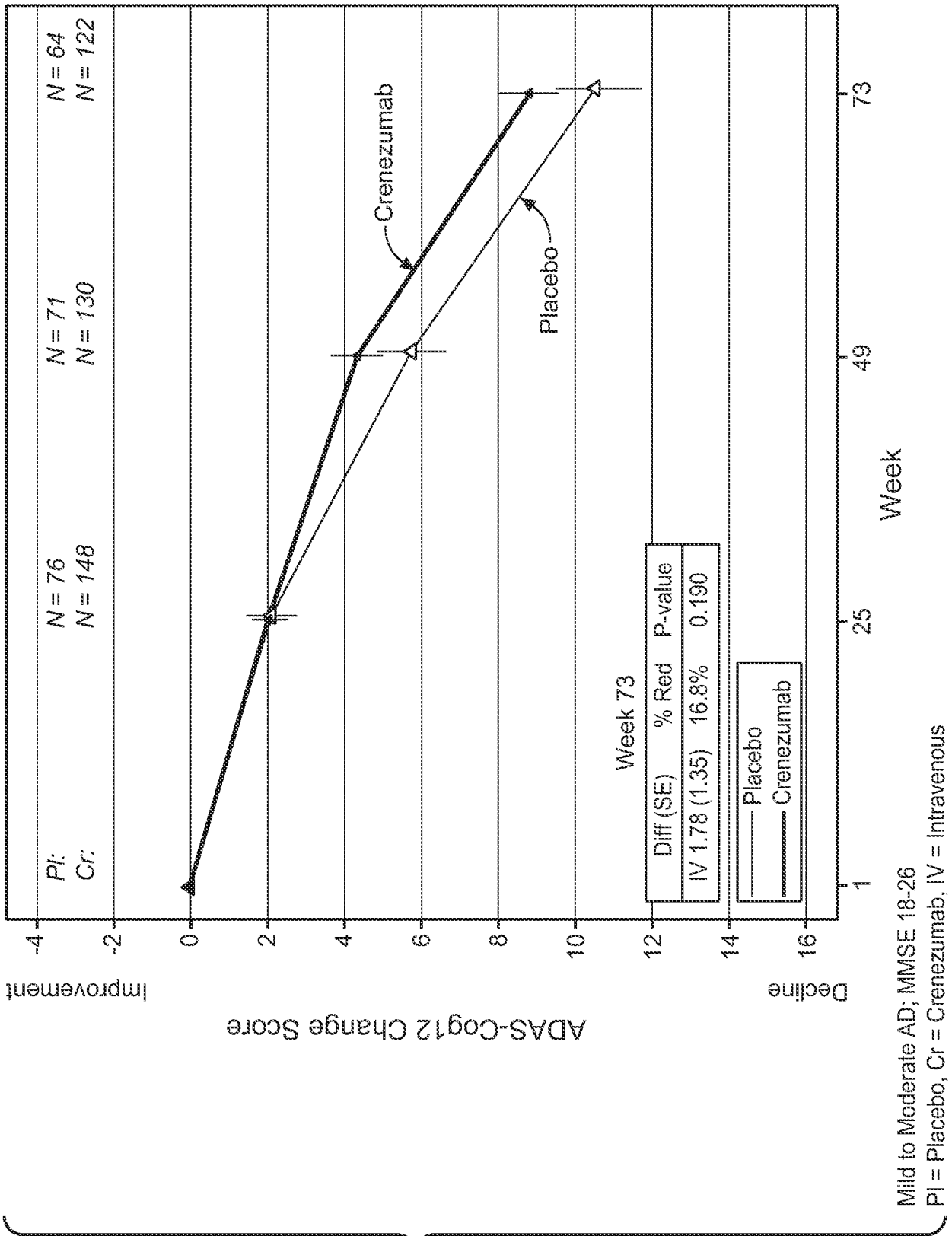
ADAS-Cog12 Change Score
Improvement
Decline
-4
-2
0
2
4
6
8
10
12
14
16
Week
1
25
49
73
Placebo
Crenezumab
Pl: N = 76 N = 71 N = 64
Cr: N = 148 N = 130 N = 122
Week 73
Diff (SE) % Red P-value
IV 1.78 (1.35) 16.8% 0.190
Placebo
Crenezumab
Mild to Moderate AD; MMSE 18-26
Pl = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 8

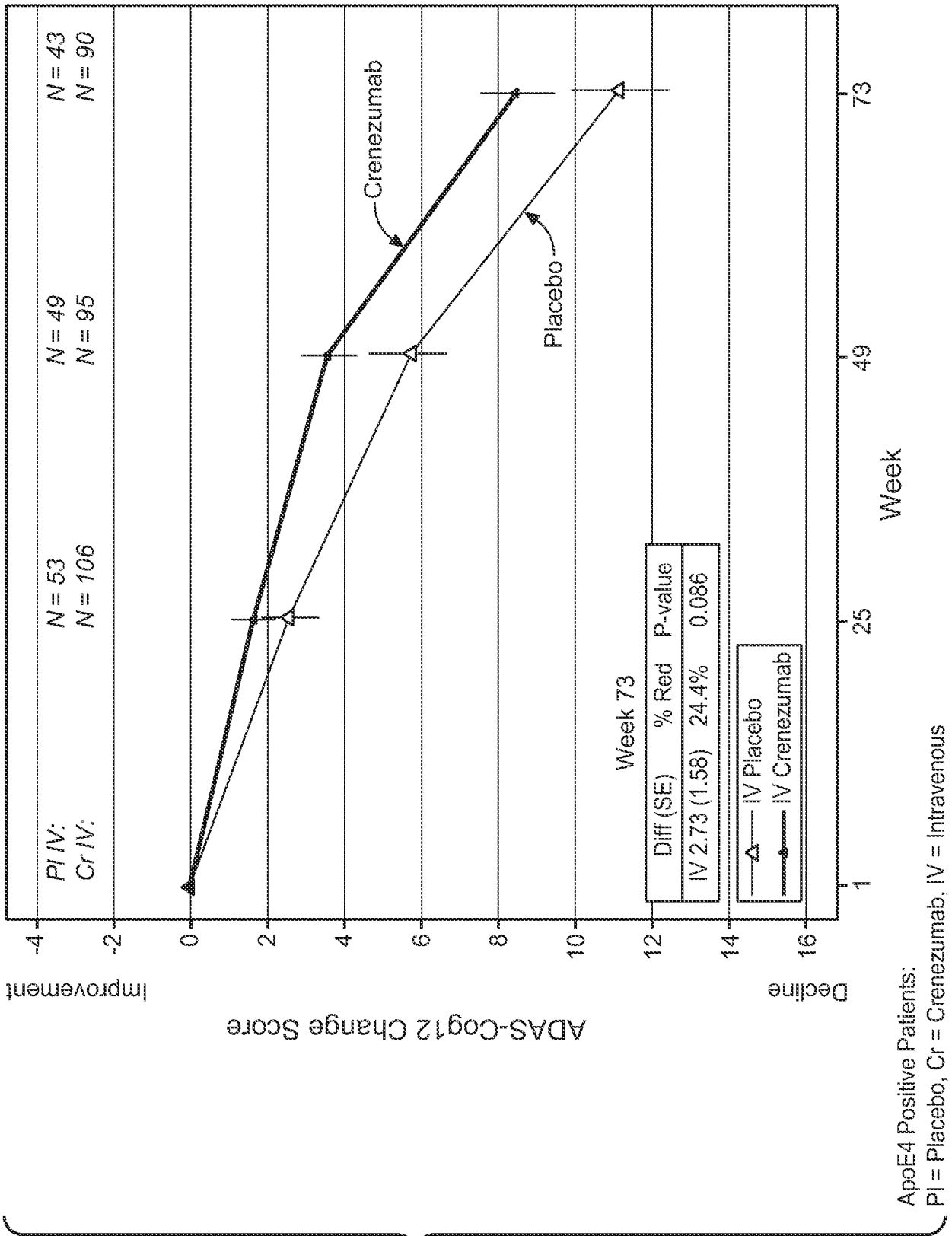
ADAS-Cog12 Change Score
Improvement
Decline
-4
-2
0
2
4
6
8
10
12
14
16
Week
1
25
49
73
Crenezumab
Placebo
PI IV: N = 53 N = 49 N = 43
Cr IV: N = 106 N = 95 N = 90
Week 73
Diff (SE) % Red P-value
IV 2.73 (1.58) 24.4% 0.086
IV Placebo
IV Crenezumab
ApoE4 Positive Patients:
PI = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 9

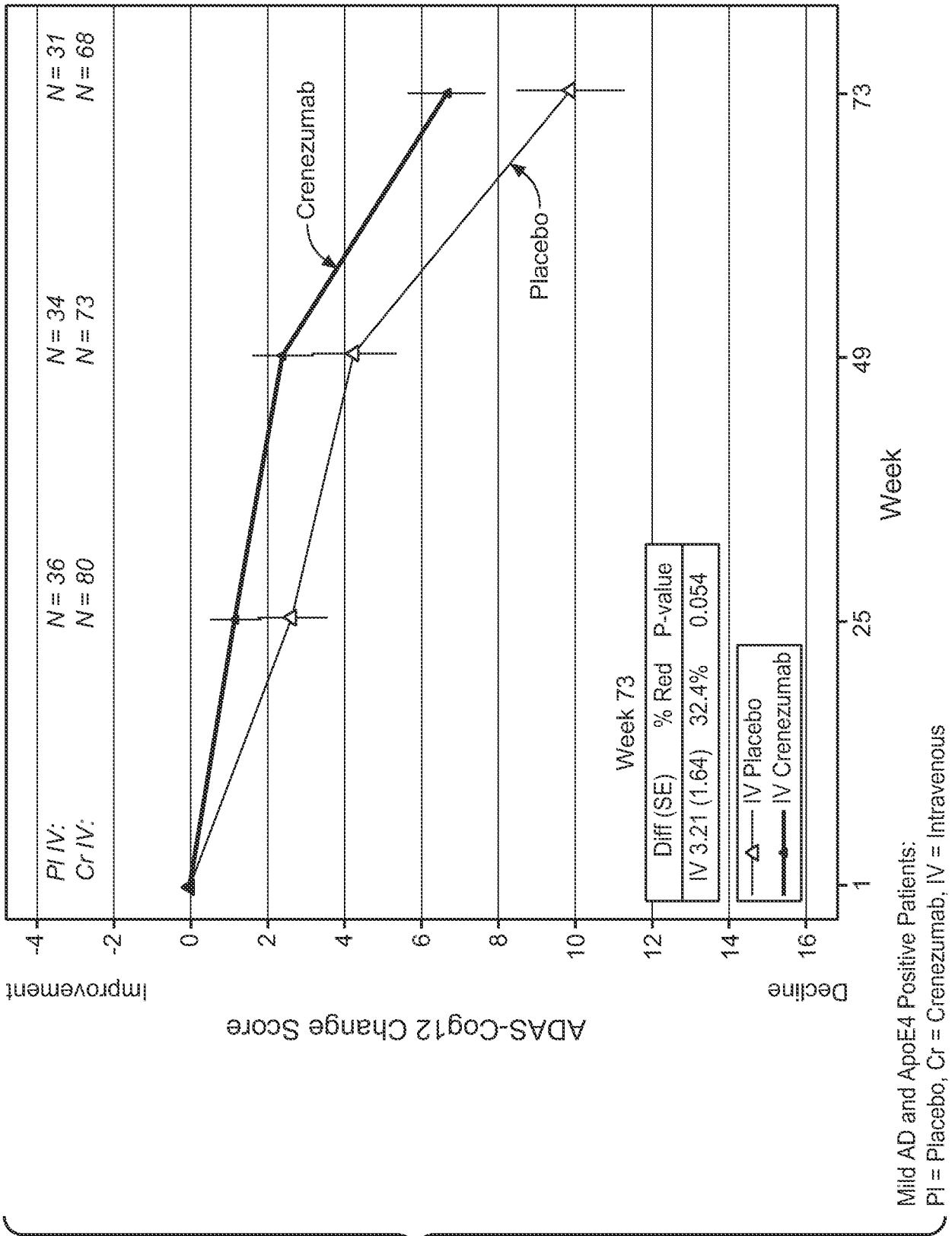
PI IV: N = 36 N = 34 N = 31
Cr IV: N = 80 N = 73 N = 68
Crenezumab
Placebo
Week 73
Diff (SE) % Red P-value
IV 3.21 (1.64) 32.4% 0.054
IV Placebo
IV Crenezumab
ADAS-Cog12 Change Score
Decline
Improvement
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
Week
Mild AD and ApoE4 Positive Patients:
PI = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 10

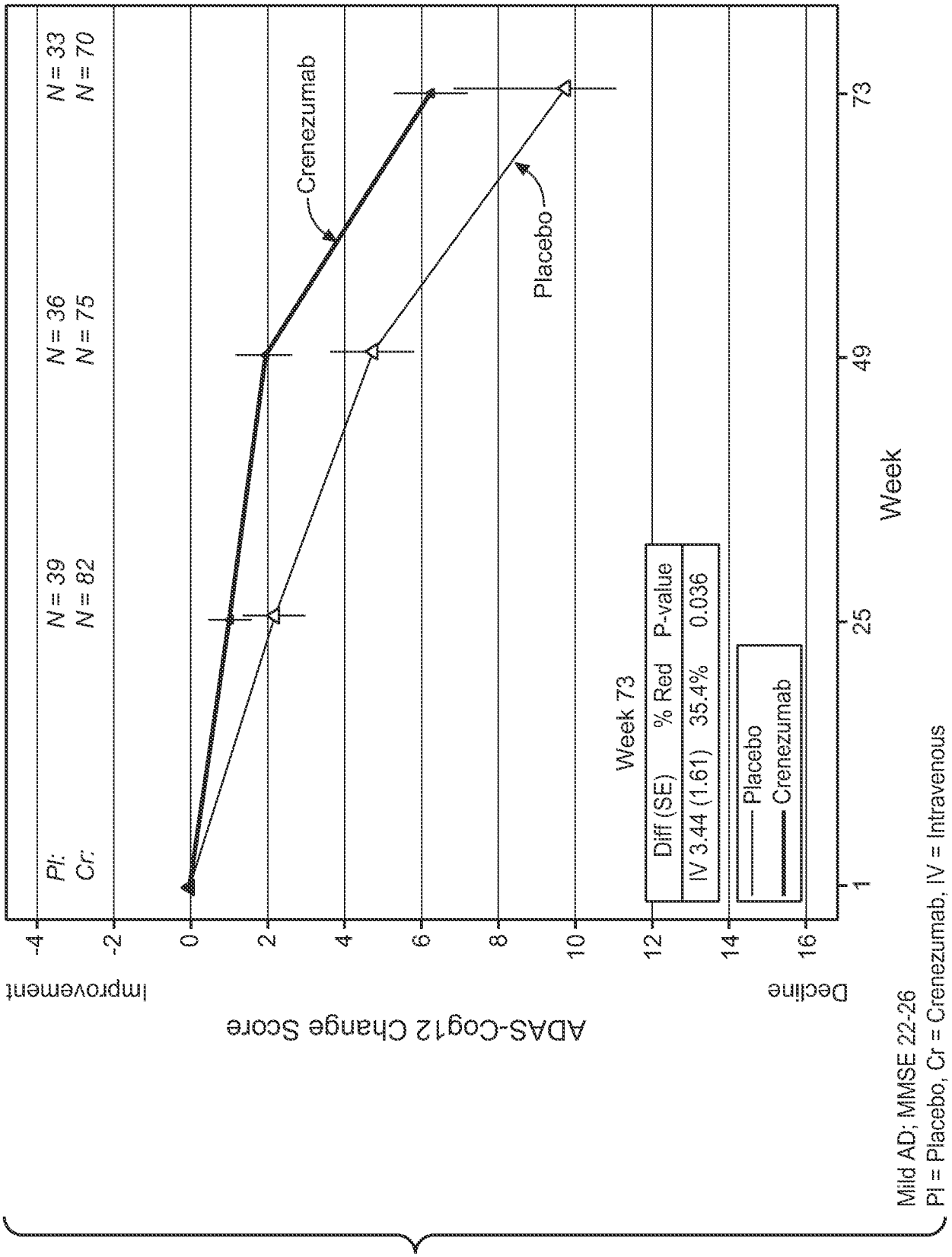
ADAS-Cog12 Change Score
Improvement
Decline
-4
-2
0
2
4
6
8
10
12
14
16
Week
1
25
49
73
Crenezumab
Placebo
Pl: N = 39 N = 36 N = 33
Cr: N = 82 N = 75 N = 70
Week 73
Diff (SE) % Red P-value
IV 3.44 (1.61) 35.4% 0.036
Placebo
Crenezumab
Mild AD; MMSE 22-26
Pl = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 11

| IV Cohort CDR-SOB: Week 73 MMRM Results on Observed Case Data | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| MMSE Range (% of MITT) | N at Week 73 | | Placebo LS Mean (SE) Change Score | Crenezumab LS Mean (SE) Change Score | Difference | | | % Reduction | ES (SD) |
| | Placebo | Crenezumab | | | LS Mean (SE) | 80% CI | P-value | | |
| 18-26 (100%) | 67 | 126 | 2.57 (0.35) | 2.48 (0.25) | 0.09 (0.43) | -0.46, 0.64 | 0.837 | 3.4% | 0.03 (2.94) |
| 19-26 (86%) | 58 | 108 | 2.65 (0.38) | 2.43 (0.28) | 0.22 (0.47) | -0.39, 0.83 | 0.641 | 8.30% | 0.07 (3.02) |
| 20-26 (74%) | 48 | 96 | 2.18 (0.40) | 2.21 (0.28) | -0.02 (0.49) | -0.66, 0.61 | 0.964 | -1.0% | -0.01 (2.91) |
| 21-26 (64%) | 40 | 85 | 2.26 (0.45) | 2.16 (0.31) | 0.10 (0.54) | -0.60, 0.81 | 0.848 | 4.6% | 0.04 (2.98) |
| 22-26 (54%) | 34 | 71 | 2.24 (0.45) | 1.80 (0.31) | 0.44 (0.55) | -0.27, 1.14 | 0.423 | 19.6% | 0.16 (2.75) |
| 23-26 (42%) | 24 | 60 | 1.88 (0.45) | 1.48 (0.28) | 0.40 (0.53) | -0.28, 1.08 | 0.449 | 21.40% | 0.18 (2.25) |
| 24-26 (31%) | 16 | 45 | 1.87 (0.45) | 1.02 (0.27) | 0.85 (0.52) | 0.16, 1.53 | 0.114 | 45.40% | 0.46 (1.85) |
| 25-26 (20%) | 11 | 30 | 1.95 (0.44) | 0.71 (0.27) | 1.24 (0.52) | 0.56, 1.92 | 0.022 | 63.60% | 0.83 (1.50) |

FIG. 12A

| | | | CDR-SOB Total | | | | CDR-SOB Judgment & Problem Solving | | | CDR-SOB Memory | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| MMSE | N (Plc) | N (Cre) | Δ (SE) | Δ % | ES (SD) | P | Δ (SE) | Δ % | P | Δ (SE) | Δ % | P |
| 18-26 | 67 | 126 | 0.09 (0.43) | 3.4% | 0.03 (2.94) | 0.837 | 0.02 (0.08) | 5.7% | 0.790 | 0.08 (0.08) | 18.2% | 0.311 |
| 20-26 | 48 | 96 | -0.02 (0.49) | -1.0% | 0.01 (2.91) | 0.964 | 0.06 (0.09) | 16.1% | 0.517 | 0.09 (0.09) | 21.7% | 0.346 |
| 22-26 | 34 | 71 | 0.44 (0.55) | 19.6% | 0.16 (2.75) | 0.423 | 0.12 (0.10) | 29.9% | 0.236 | 0.16 (0.10) | 42.7% | 0.124 |

FIG. 12B

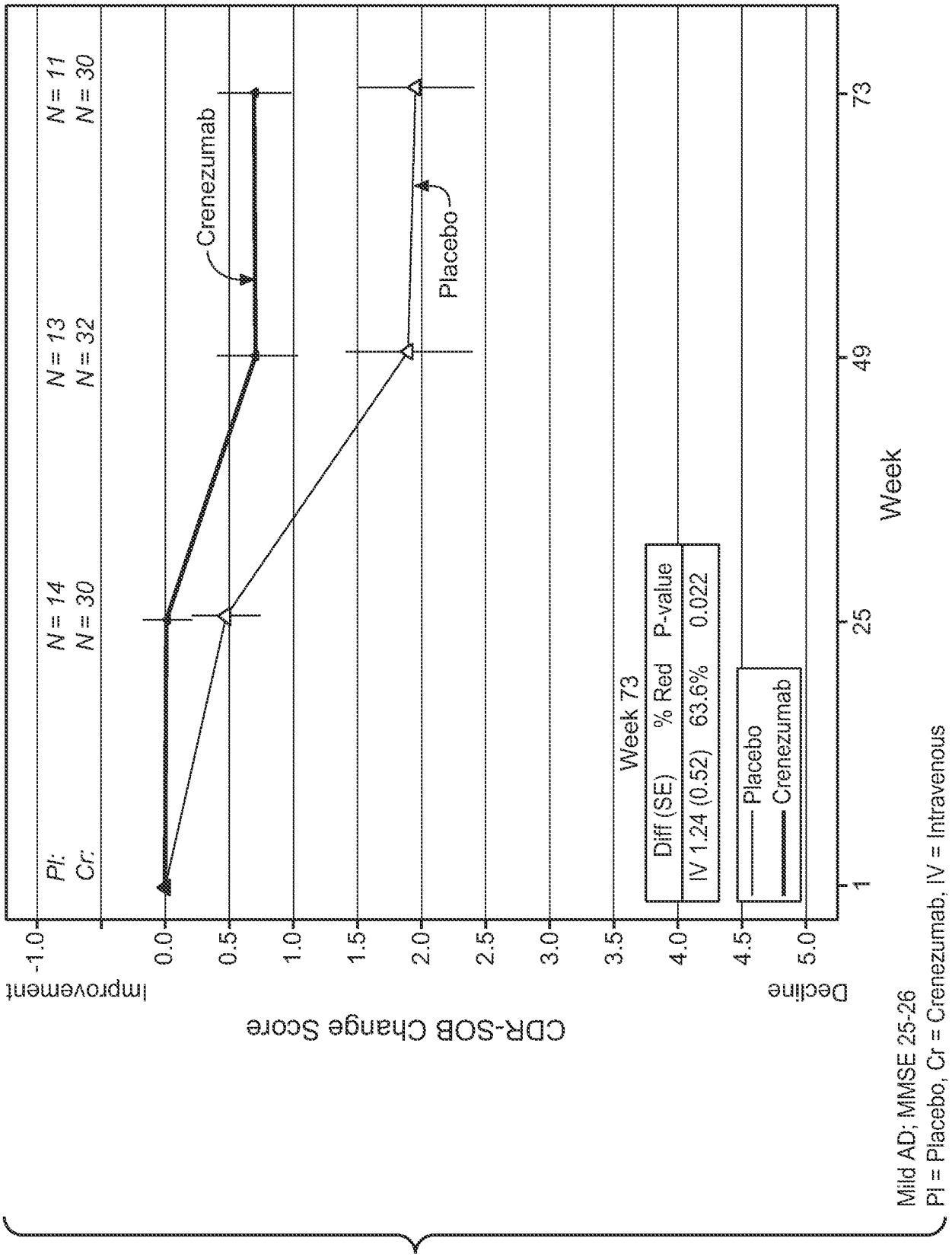
CDR-SOB Change Score
Improvement
Decline
-1.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
Week
1
25
49
73
Pl: N = 14 N = 13 N = 11
Cr: N = 30 N = 32 N = 30
Crenezumab
Placebo
Week 73
Diff (SE) % Red P-value
IV 1.24 (0.52) 63.6% 0.022
Placebo
Crenezumab
Mild AD; MMSE 25-26
Pl = Placebo, Cr = Crenezumab, IV = Intravenous

FIG. 13

| | 15 mg/kg IV q4w | |
|---|---|---|
| | Placebo | Cren |
| **Baseline** | N=17 | N=35 |
| Age | 69.8 (7.7) | 71.4 (7.1) |
| Female % | 35.3% | 68.6% |
| MMSE | 20.5 (2.2) | 20.8 (2.3) |
| MMSE 20-26 (mild) | 58.8% | 60.0% |
| APOE4 Carriers | 70.6% | 68.6% |
| ADAS-Cog12 | 34.51 (11.13) | 31.21 (9.88) |
| CDR-SOB | 5.9 (1.9) | 4.9 (2.0) |
| ADCS-ADL | 64.5 (8.2) | 66.8 (7.4) |
| SUVR (Cerebellar Gray Ref) | 1.77 (0.31) | 1.74 (0.28) |
| AChEI and/or Memantine Use | 82.4% | 91.4% |
| | | |
| **Completed Treatment** | 10 (58.8%) | 21 (60.0%) |
| Discontinued Study | 7 (41.2%) | 14 (40.0%) |
| Death | 0 | 2 (5.7%) |
| Adverse Event | 3 (17.6%) | 1 (2.9%) |
| Withdrawal by Subject | 3 (17.6%) | 8 (22.9%) |
| Other | 1 (5.9%) | 3 (8.6%) |

*FIG. 14A*

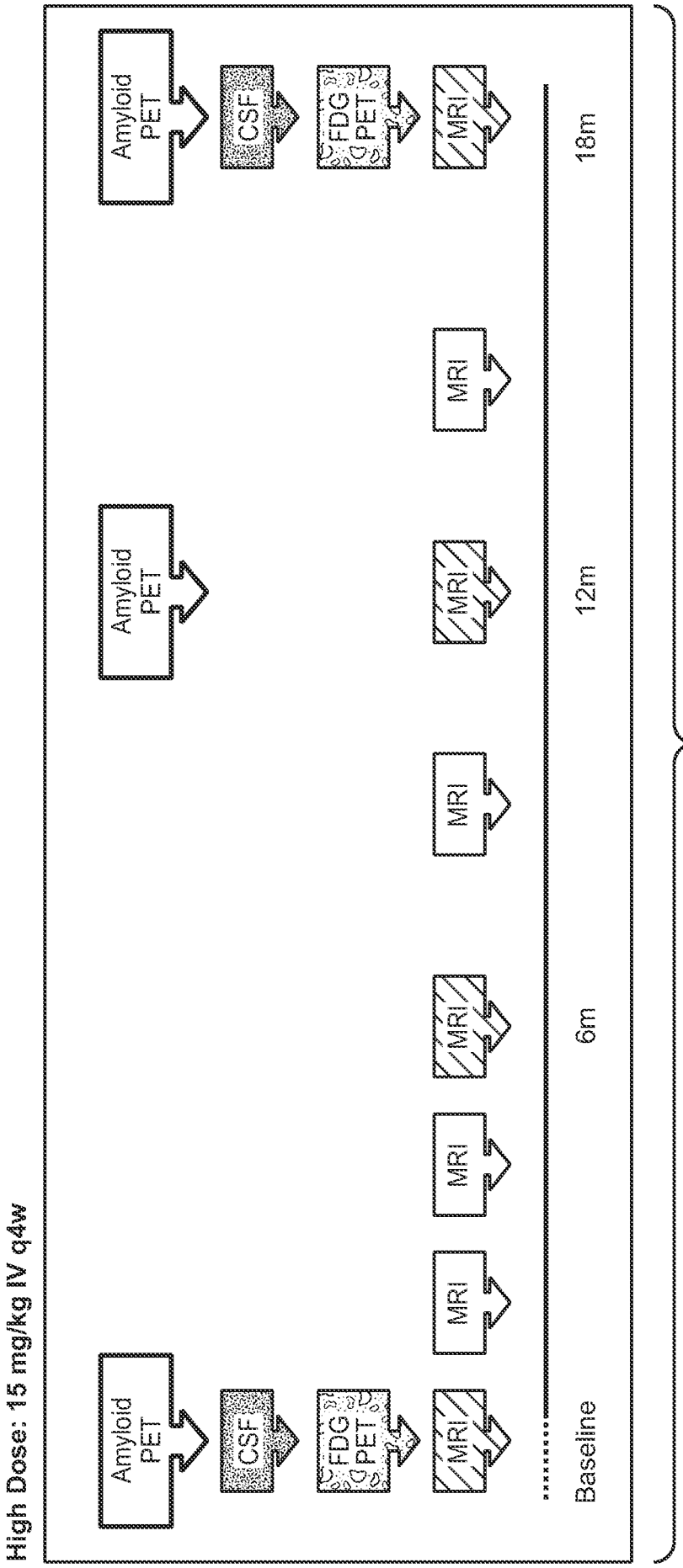
High Dose: 15 mg/kg IV q4w
Amyloid PET
CSF
FDG PET
MRI
MRI
MRI
MRI
MRI
MRI
MRI
Amyloid PET
Amyloid PET
CSF
FDG PET
MRI
MRI
MRI
MRI
Baseline
6m
12m
18m

FIG. 14B

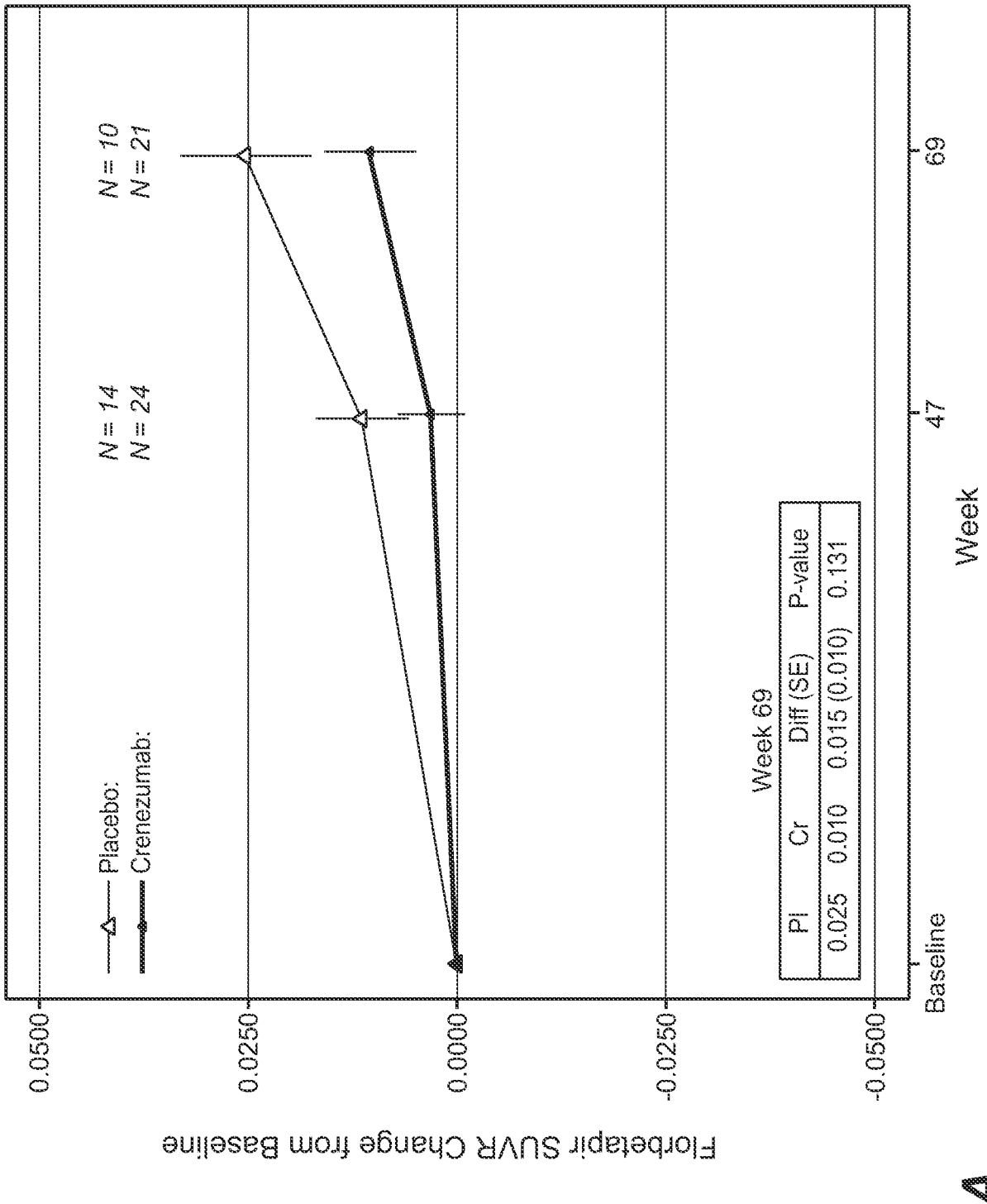
Placebo:
Crenezumab:
N = 14
N = 24
N = 10
N = 21
Week 69
PI Cr Diff (SE) P-value
0.025 0.010 0.015 (0.010) 0.131
0.0500
0.0250
0.0000
-0.0250
-0.0500
Baseline
47
69
Week
Florbetapir SUVR Change from Baseline

FIG. 15A

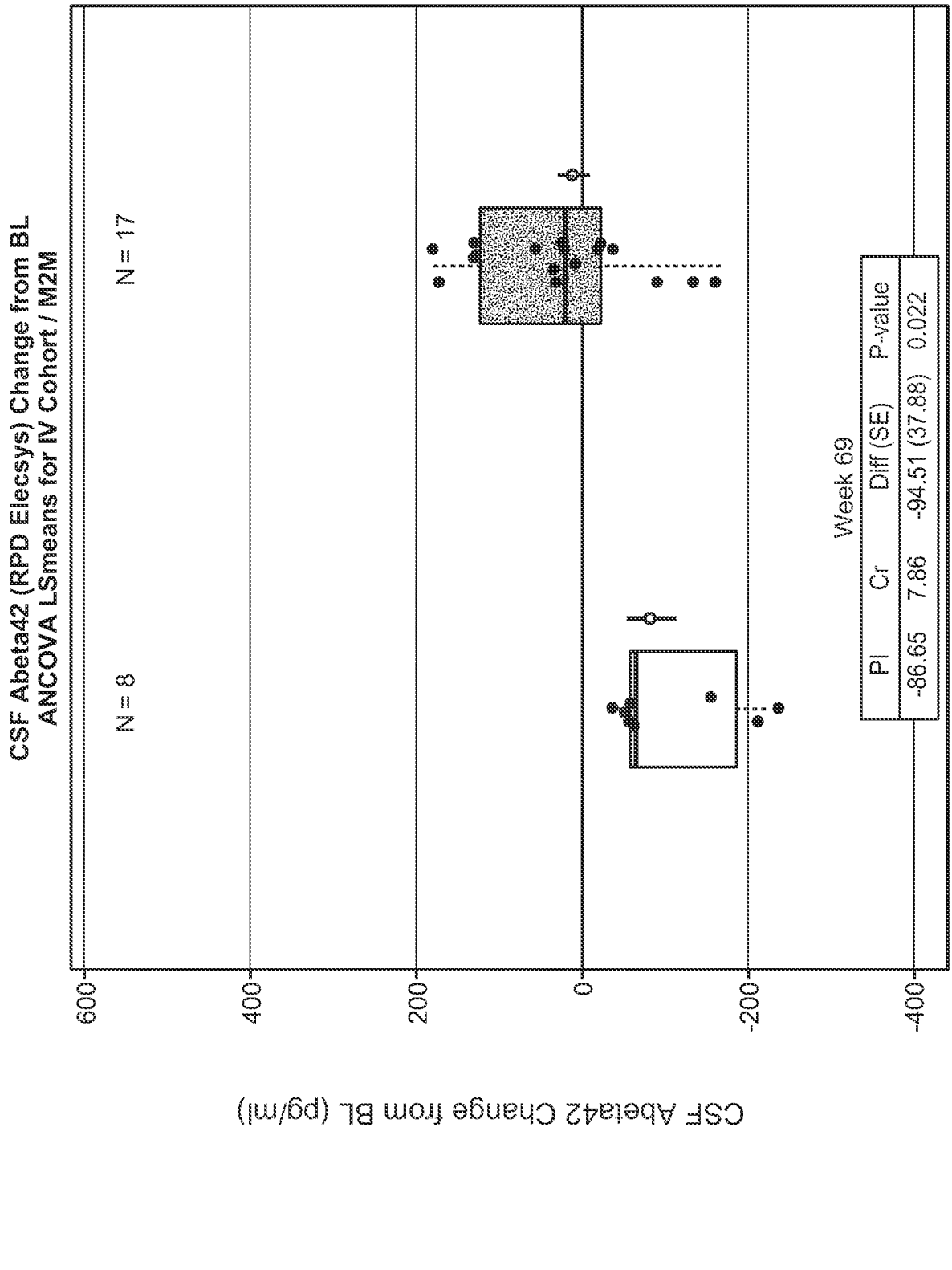
CSF Abeta42 (RPD Elecsys) Change from BL
ANCOVA LSmeans for IV Cohort / M2M
N = 8
N = 17
600
400
200
0
-200
-400
CSF Abeta42 Change from BL (pg/ml)
Week 69
Pl Cr Diff (SE) P-value
-86.65 7.86 -94.51 (37.88) 0.022
Pbo
Week 69
Cren

FIG. 15B

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/014758

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/00 A61P25/28 C07K16/18
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
A61P A61K C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, WPI Data, BIOSIS, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | RACHELLE S. DOODY ET AL: "Phase 3 Trials of Solanezumab for Mild-to-Moderate Alzheimer's Disease", NEW ENGLAND JOURNAL OF MEDICINE, vol. 370, no. 4, 23 January 2014 (2014-01-23), pages 311-321, XP055183832, ISSN: 0028-4793, DOI: 10.1056/NEJMoa1312889 page 317, left-hand column, lines 6-15; tables 1, 4, 5 page 319, left-hand column, line 10 - page 319, right-hand column, line 37 ----- -/-- | 1-116 |

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 6 May 2015 | 22/05/2015 |

| Name and mailing address of the ISA/ | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040,<br>Fax: (+31-70) 340-3016 | Cilensek, Zoran |

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X | NIELS D PRINS ET AL: "Treating Alzheimer?s disease with monoclonal antibodies: current status and outlook for the future", ALZHEIMERS RES THER, BIOMED CENTRAL LTD, LONDON, UK, vol. 5, no. 6, 11 November 2013 (2013-11-11), page 56, XP021193607, ISSN: 1758-9193, DOI: 10.1186/ALZRT220 page 4, left-hand column, paragraph 1 - page 4, right-hand column, paragraph 1; table 1 | 1-116 |
| X | O. ADOLFSSON ET AL: "An Effector-Reduced Anti- -Amyloid (A ) Antibody with Unique A Binding Properties Promotes Neuroprotection and Glial Engulfment of A", JOURNAL OF NEUROSCIENCE, vol. 32, no. 28, 11 July 2012 (2012-07-11) , pages 9677-9689, XP055097358, ISSN: 0270-6474, DOI: 10.1523/JNEUROSCI.4742-11.2012 page 9686, left-hand column, paragraph 1-2; figures 1-7 | 1-116 |
| X | WO 2012/016173 A2 (AC IMMUNE SA [CH]; GENENTECH INC [US]; PFEIFER ANDREA [CH]; MUHS ANDRE) 2 February 2012 (2012-02-02) paragraph [0191] - paragraph [0205] | 1-116 |
| X | Charlotte Jago: "Alzheimer's Disease: One Year Later", , 3 February 2014 (2014-02-03), XP055183816, Retrieved from the Internet: URL:http://lsconnect.thomsonreuters.com/alzheimers-disease-year-later/ [retrieved on 2015-04-17] page 5 | 1-116 |
| X,P | "Crenezumab Phase II cognition data in Alzheimer's disease presented", , 16 July 2014 (2014-07-16), XP055183813, Retrieved from the Internet: URL:http://www.acimmune.com/content/img/pages/ACImmune_crenezumab_phase2_ENGLISH_20140716_final.pdf [retrieved on 2015-04-17] page 1, paragraph 1 page 2, paragraphs 2,3 | 1-116 |

-/--

# INTERNATIONAL SEARCH REPORT

International application No
PCT/US2015/014758

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| A | WO 2006/066049 A2 (NEURALAB LTD; WYETH CORP [US]; BASI GURIQ [US]; JACOBSON JACK STEVEN [) 22 June 2006 (2006-06-22) figure 3; examples 1-19 ----- | 1-116 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2015/014758

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| WO 2012016173 A2 | 02-02-2012 | AU 2011282536 A1 | 21-02-2013 |
| | | CA 2806909 A1 | 02-02-2012 |
| | | CN 103179981 A | 26-06-2013 |
| | | EP 2598882 A2 | 05-06-2013 |
| | | JP 2013538796 A | 17-10-2013 |
| | | KR 20130136968 A | 13-12-2013 |
| | | RU 2013108841 A | 10-09-2014 |
| | | SG 187173 A1 | 28-02-2013 |
| | | US 2012064065 A1 | 15-03-2012 |
| | | WO 2012016173 A2 | 02-02-2012 |
| WO 2006066049 A2 | 22-06-2006 | AR 051528 A1 | 17-01-2007 |
| | | EP 1838854 A2 | 03-10-2007 |
| | | ES 2396555 T3 | 22-02-2013 |
| | | PE 11522006 A1 | 13-10-2006 |
| | | US 2006165682 A1 | 27-07-2006 |
| | | UY 29282 A1 | 30-06-2006 |
| | | WO 2006066049 A2 | 22-06-2006 |

(19)中华人民共和国国家知识产权局

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62/010,259 2014.06.10 US
62/081,992 2014.11.19 US

(85)PCT国际申请进入国家阶段日
2016.09.29

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罗伯特·保罗 迈克尔·沃德

(74)专利代理机构 北京安信方达知识产权代理有限公司 11262
代理人 郑霞

(51)Int.Cl.
*A61K 39/00*(2006.01)
*A61P 25/28*(2006.01)
*C07K 16/18*(2006.01)

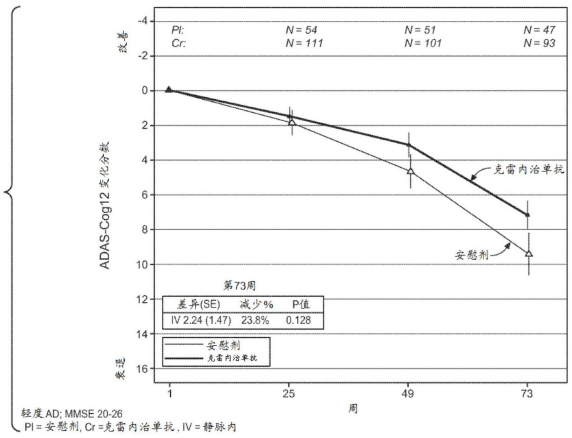
权利要求书7页 说明书46页
序列表5页 附图15页

(54)发明名称

治疗阿尔茨海默氏病的方法

(57)摘要

提供了治疗罹患轻到中度AD的患者(包括ApoE4阳性患者和罹患轻度AD的患者)中的阿尔茨海默氏病(AD)的方法。

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1.一种减少被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的功能或认知能力衰退的方法,其包括向罹患早期或轻到中度AD的患者施用能有效减缓所述患者中的功能或认知能力衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体,所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

2.根据权利要求1所述的方法,其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

3.根据权利要求1所述的方法,其中所述抗体是IgG4抗体。

4.根据权利要求2或3所述的方法,其中所述抗体包含六个高变区(HVR),其中:

(i)HVR-H1是SEQ ID NO:2;

(ii)HVR-H2是SEQ ID NO:3;

(iii)HVR-H3是SEQ ID NO:4;

(iv)HVR-L1是SEQ ID NO:6;

(v)HVR-L2是SEQ ID NO:7;且

(vi)HVR-L3是SEQ ID NO:8。

5.根据权利要求4所述的方法,其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

6.根据权利要求5所述的方法,其中所述抗体是克雷内治单抗。

7.根据前述权利要求中任一项所述的方法,其中通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分来评估认知能力的衰退,任选地,其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30%、至少35%、至少40%或至少45%。

8.根据权利要求7所述的方法,其中所述患者为ApoE4阳性。

9.根据权利要求7所述的方法,其中所述患者罹患轻度AD。

10.根据权利要求7所述的方法,其中所述患者罹患早期AD。

11.根据权利要求1至8中任一项所述的方法,其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

12.根据权利要求11所述的方法,其中所述患者具有22和26之间的MMSE。

13.根据前述权利要求中任一项所述的方法,其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

14.根据权利要求13所述的方法,其中以至少15mg/kg的剂量施用所述抗体。

15.根据权利要求14所述的方法,其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

16.根据权利要求13或14所述的方法,其中经由静脉内注射施用所述抗体。

17.根据权利要求13至16中任一项所述的方法,其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

18.一种治疗早期或轻到中度AD而不增加不良事件的风险的方法,其包括向被诊断为早期或轻到中度AD的患者施用能有效治疗所述AD而不增加治疗出现的不良事件的风险的

用量的人源化单克隆抗Aβ抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内，其中所述不良事件选自：(i)淀粉样蛋白相关成像异常-水肿(ARIA-E)和(ii)淀粉样蛋白相关成像异常-出血(ARIA-H)。

19.根据权利要求18所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

20.根据权利要求18所述的方法，其中所述抗体是IgG4抗体。

21.根据权利要求19所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

(i)HVR-H1是SEQ ID NO:2；

(ii)HVR-H2是SEQ ID NO:3；

(iii)HVR-H3是SEQ ID NO:4；

(iv)HVR-L1是SEQ ID NO:6；

(v)HVR-L2是SEQ ID NO:7；且

(vi)HVR-L3是SEQ ID NO:8。

22.根据权利要求21所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

23.根据权利要求22所述的方法，其中所述抗体是克雷内治单抗。

24.根据权利要求18至23中任一项所述的方法，其中所述患者为ApoE4阳性。

25.根据权利要求18至23中任一项所述的方法，其中所述不良事件为ARIA-E。

26.根据权利要求25所述的方法，其中如果检测到治疗出现的ARIA-E，则停止施用所述抗体并且任选地施用针对ARIA-E的治疗。

27.根据权利要求26所述的方法，其进一步包括在解决所述ARIA-E后恢复施用所述抗体，其中所述抗体以低于停止施用前的剂量施用。

28.根据权利要求18所述的方法，其中如果在用所述抗体进行治疗期间在所述患者中检测到一个或多个新的ARIA-E，则不再施用抗体，并且任选地向所述患者施用皮质类固醇。

29.根据权利要求28所述的方法，其中所述患者为ApoE4阳性。

30.一种减少被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的功能或认知能力衰退的方法，其包括向罹患早期或轻到中度AD的ApoE4阳性患者施用能有效减缓所述患者中的功能或认知能力衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

31.根据权利要求30所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

32.根据权利要求30所述的方法，其中所述抗体是IgG4抗体。

33.根据权利要求31或32所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

(i)HVR-H1是SEQ ID NO:2；

(ii)HVR-H2是SEQ ID NO:3；

(iii)HVR-H3是SEQ ID NO:4；

(iv)HVR-L1是SEQ ID NO:6；

(v)HVR-L2是SEQ ID NO:7；且

(vi)HVR-L3是SEQ ID NO:8。

34.根据权利要求33所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

35.根据权利要求34所述的方法，其中所述抗体是克雷内治单抗。

36.根据权利要求30至35中任一项所述的方法，其中通过使用ADAS-Cog12、ADAS-Cog13或ADAS-Cog14测试确定在施用所述抗体之前和之后的患者评分来评估认知能力的衰退，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

37.根据权利要求36所述的方法，其中所述患者具有轻度AD。

38.根据权利要求36所述的方法，其中所述患者具有早期AD。

39.根据权利要求30至37中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

40.根据权利要求39所述的方法，其中所述患者具有22和26之间的MMSE评分。

41.根据权利要求30至39中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

42.根据权利要求41所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

43.根据权利要求42所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

44.根据权利要求41或42所述的方法，其中经由静脉内注射施用所述抗体。

45.根据权利要求41至44中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

46.一种治疗早期或轻到中度AD而不增加不良事件的风险的方法，其包括向被诊断为早期或轻到中度AD的ApoE4阳性患者施用能有效治疗所述AD而不增加治疗出现的不良事件的风险的用量的人源化单克隆抗Aβ抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内，其中所述不良事件选自：(i)淀粉样蛋白相关成像异常-水肿(ARIA-E)和(ii)淀粉样蛋白相关成像异常-出血(ARIA-H)。

47.根据权利要求46所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

48.根据权利要求46所述的方法，其中所述抗体是IgG4抗体。

49.根据权利要求47所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

(i)HVR-H1是SEQ ID NO:2；

(ii)HVR-H2是SEQ ID NO:3；

(iii)HVR-H3是SEQ ID NO:4；

(iv)HVR-L1是SEQ ID NO:6；

(v)HVR-L2是SEQ ID NO:7；且

(vi)HVR-L3是SEQ ID NO:8。

50.根据权利要求49所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

51.根据权利要求50所述的方法，其中所述抗体是克雷内治单抗。

52.根据权利要求46至51中任一项所述的方法，其中所述不良事件为ARIA-E。

53.根据权利要求52所述的方法，其中如果检测到治疗出现的ARIA-E，则停止施用所述抗体并且任选地施用针对ARIA-E的治疗。

54.根据权利要求53所述的方法，其进一步包括在解决所述ARIA-E后恢复施用所述抗体，任选地包括以低于停止施用前的剂量恢复施用所述抗体。

55.根据权利要求46所述的方法，其中如果在用所述抗体进行治疗期间在所述患者中检测到一个或多个新的ARIA-E，则不再施用抗体，并且任选地向所述患者施用皮质类固醇。

56.根据前述权利要求中任一项所述的方法，其中同时用选自由以下所组成的组的一种或多种药剂治疗所述患者：特异性结合标靶的治疗剂；胆碱酯酶抑制剂；NMDA受体拮抗剂；单胺耗竭剂；甲磺酸二氢麦角碱；抗胆碱能抗帕金森病剂；多巴胺能抗帕金森病剂；丁苯喹嗪；抗炎剂；激素；维生素；二甲弗林；高牛磺酸；血清素受体活性调节剂；干扰素和糖皮质激素；除克雷内治单抗以外的抗Aβ抗体；抗生素；抗病毒剂。

57.根据权利要求56所述的方法，其中所述药剂是胆碱酯酶抑制剂。

58.根据权利要求57所述的方法，其中所述胆碱酯酶抑制剂选自由加兰他敏、多奈哌齐、卡巴拉汀和他克林组成的组。

59.根据权利要求56所述的方法，其中所述药剂是NMDA受体拮抗剂。

60.根据权利要求59所述的方法，其中所述NMDA受体拮抗剂是美金刚胺或其盐。

61.根据权利要求56所述的方法，其中所述药剂是特异性结合于标靶的治疗剂且所述标靶选自由以下所组成的组：β分泌酶、tau、早老素、淀粉样前体蛋白或其部分、淀粉样β肽或其低聚物或原纤维、死亡受体6(DR6)、晚期糖基化终产物的受体(RAGE)、帕金蛋白和亨廷顿蛋白。

62.根据权利要求56所述的方法，其中所述药剂是单胺耗竭剂，任选为丁苯喹嗪。

63.根据权利要求56所述的方法，其中所述药剂是选自由以下所组成的组的抗胆碱能抗帕金森病剂：普环啶、苯海拉明、三己芬迪、苯托品、比哌立登和苯海索。

64.根据权利要求56所述的方法，其中所述药剂是选自由以下所组成的组的多巴胺能抗帕金森病剂：恩他卡朋、司来吉兰、普拉克索、溴隐亭、罗替戈汀、司来吉兰、罗匹尼罗、雷沙吉兰、阿朴吗啡、卡比多巴、左旋多巴、培高利特、托卡朋和金刚烷胺。

65.根据权利要求56所述的方法，其中所述药剂是选自由非类固醇类抗炎药和吲哚美辛组成的组的抗炎剂。

66.根据权利要求56所述的方法，其中所述药剂是选自由雌激素、孕酮和亮丙瑞林组成的组的激素。

67.根据权利要求56所述的方法，其中所述药剂是选自由叶酸和烟酰胺组成的组的维生素。

68.根据权利要求56所述的方法，其中所述药剂是高牛磺酸，其为3-氨基丙磺酸或3APS。

69.根据权利要求56所述的方法，其中所述药剂是扎利罗登。

70.一种减缓被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的临床衰退的方法，其包括向罹患早期或轻到中度AD的患者施用能有效减缓所述患者中的衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)

的残基13和24内。

71.根据权利要求70所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

72.根据权利要求70所述的方法，其中所述抗体是IgG4抗体。

73.根据权利要求71或72所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

(i)HVR-H1是SEQ ID NO:2；

(ii)HVR-H2是SEQ ID NO:3；

(iii)HVR-H3是SEQ ID NO:4；

(iv)HVR-L1是SEQ ID NO:6；

(v)HVR-L2是SEQ ID NO:7；且

(vi)HVR-L3是SEQ ID NO:8。

74.根据权利要求73所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

75.根据权利要求74所述的方法，其中所述抗体是克雷内治单抗。

76.根据权利要求70至75中任一项所述的方法，其进一步包括减缓认知能力的衰退，其中所述认知能力的衰退是通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分来评估，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

77.根据权利要求76所述的方法，其中所述患者为ApoE4阳性。

78.根据权利要求76所述的方法，其中所述患者罹患轻度AD。

79.根据权利要求76所述的方法，其中所述患者罹患早期AD。

80.根据权利要求70至78中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

81.根据权利要求80所述的方法，其中所述患者具有22和26之间的MMSE评分。

82.根据权利要求70至80中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

83.根据权利要求82所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

84.根据权利要求83所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

85.根据权利要求82或83所述的方法，其中经由静脉内注射施用所述抗体。

86.根据权利要求82至85中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

87.一种治疗受试者中的早期或轻度AD的方法，其包括向罹患早期或轻度AD的患者施用能有效治疗所述AD的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

88.根据权利要求87所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形

式。

89.根据权利要求87所述的方法，其中所述抗体是IgG4抗体。

90.根据权利要求88或89所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

(i)HVR-H1是SEQ ID NO:2；

(ii)HVR-H2是SEQ ID NO:3；

(iii)HVR-H3是SEQ ID NO:4；

(iv)HVR-L1是SEQ ID NO:6；

(v)HVR-L2是SEQ ID NO:7；且

(vi)HVR-L3是SEQ ID NO:8。

91.根据权利要求90所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

92.根据权利要求91所述的方法，其中所述抗体是克雷内治单抗。

93.根据权利要求87至92中任一项所述的方法，其中所述用量能有效减少认知能力的衰退，所述认知能力的衰退是通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分来评估，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

94.根据权利要求93所述的方法，其中所述患者为ApoE4阳性。

95.根据权利要求87至94中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

96.根据权利要求95所述的方法，其中所述患者具有22和26之间的MMSE评分。

97.根据权利要求87至95中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

98.根据权利要求97所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

99.根据权利要求98所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

100.根据权利要求97或98所述的方法，其中经由静脉内注射施用所述抗体。

101.根据权利要求97至100中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

102.根据权利要求70至101中任一项所述的方法，其中同时用选自由以下所组成的组的一种或多种药剂治疗所述患者：特异性结合标靶的治疗剂；胆碱酯酶抑制剂；NMDA受体拮抗剂；单胺耗竭剂；甲磺酸二氢麦角碱；抗胆碱能抗帕金森病剂；多巴胺能抗帕金森病剂；丁苯喹嗪；抗炎剂；激素；维生素；二甲弗林；高牛磺酸；血清素受体活性调节剂；干扰素和糖皮质激素；抗Aβ抗体；抗生素；抗病毒剂。

103.根据权利要求102所述的方法，其中所述药剂是胆碱酯酶抑制剂。

104.根据权利要求103所述的方法，其中所述胆碱酯酶抑制剂选自由加兰他敏、多奈哌齐、卡巴拉汀和他克林组成的组。

105.根据权利要求102所述的方法，其中所述药剂是NMDA受体拮抗剂。

106.根据权利要求105所述的方法，其中所述NMDA受体拮抗剂是美金刚胺或其盐。

107.根据权利要求102所述的方法，其中所述药剂是特异性结合标靶的治疗剂且所述标靶选自由以下所组成的组：β分泌酶、tau、早老素、淀粉样前体蛋白或其部分、淀粉样β肽或其低聚物或原纤维、死亡受体6(DR6)、晚期糖基化终产物的受体(RAGE)、帕金蛋白和亨廷顿蛋白。

108.根据权利要求102所述的方法，其中所述药剂是单胺耗竭剂，任选为丁苯喹嗪。

109.根据权利要求102所述的方法，其中所述药剂是选自由以下所组成的组的抗胆碱能抗帕金森病剂：普环啶、苯海拉明、三己芬迪、苯托品、比哌立登和苯海索。

110.根据权利要求102所述的方法，其中所述药剂是选自由以下所组成的组的多巴胺能抗帕金森病剂：恩他卡朋、司来吉兰、普拉克索、溴隐亭、罗替戈汀、司来吉兰、罗匹尼罗、雷沙吉兰、阿朴吗啡、卡比多巴、左旋多巴、培高利特、托卡朋和金刚烷胺。

111.根据权利要求102所述的方法，其中所述药剂是选自由非类固醇类抗炎药和吲哚美辛组成的组的抗炎剂。

112.根据权利要求102所述的方法，其中所述药剂是选自由雌激素、孕酮和亮丙瑞林组成的组的激素。

113.根据权利要求102所述的方法，其中所述药剂是选自由叶酸和烟酰胺组成的组的维生素。

114.根据权利要求102所述的方法，其中所述药剂是高牛磺酸，其为3-氨基丙磺酸或3APS。

115.根据权利要求102所述的方法，其中所述药剂是扎利罗登。

116.根据权利要求102所述的方法，其中所述药剂是除克雷内治单抗以外的抗Aβ抗体。

# 治疗阿尔茨海默氏病的方法

[0001] 相关申请的交叉引用

[0002] 本申请要求2014年2月8日提交的美国临时申请第61/937,472号、2014年3月27日提交的美国临时申请第61/971,479号、2014年6月10日提交的美国临时申请第62/010,259号和2014年11月9日提交的美国临时申请第62/081,992号的权益，上述申请的内容通过引用整体并入本文中。

## 发明领域

[0003] 提供了使用靶向淀粉样蛋白β的抗体治疗罹患轻到中度阿尔茨海默氏病(Alzheimer's Disease)的患者的方法。

[0004] 发明背景

[0005] 阿尔茨海默氏病(AD)是痴呆的最常见病因，在美国影响估计450万个体且在全世界影响2660万个体(Hebert等人，Arch.Neurol.2003;60:1119-22;Brookmeyer等人，Alzheimers Dement.2007;3:186-91)。该疾病的病理特征在于大脑中细胞外β淀粉样蛋白(“Aβ”)斑块的累积和细胞内神经纤维缠结。通过AD的神经病学和神经精神病学迹象和症状的临床评价并排除痴呆的其它病因进行诊断。AD通常通过简单认知筛选检查(简易智力状态检查(“MMSE”))分为轻度、中度和严重阶段。抑制乙酰胆碱酯酶(“AChE”)活性或拮抗大脑中的N-甲基-D天冬氨酸盐受体的批准医药疗法可以暂时改善一些患者中的AD症状，但不能改变所述疾病的进展(Cummings,N.Engl.J.Med.2004;351:56-67)。

[0006] 现已有文献充分记录早发和晚发家族性AD的遗传因子。ApoE4等位基因与晚发家族性和散发性AD密切相关，其中在患有AD的患者中报告等位基因频率为50％-65％，这大约是普通群体和其它神经病症中的三倍(Saunders等人，Neurology 1993;43:1467-72;Prekumar等人，Am.J.Pathol.1996;148:2083-95)。除AD以外，ApoE4等位基因还涉及其它淀粉样蛋白形成病症(包括脑淀粉样血管病(“CAA“))(Prekumar等人，Am.J.Pathol.1996;148:2083-95)。因此，携带ApoE4等位基因的患者可以代表病因学上不同的AD患者的群体。

[0007] 细胞外淀粉样蛋白斑在大脑中的沉积是AD中的标志性病理发现，首次由Alois Alzheimer在1906年报告。这些淀粉样蛋白斑主要包含Aβ肽(Haass和Selkoe,Nature 2007;8:656-67)，它是由淀粉样前体蛋白(“APP”)通过β-和γ-分泌酶活性的相继裂解生成。Aβ，具体地说它的低聚形式，对神经元具有毒性，且据信是AD的原因。降低大脑中Aβ含量的疗法可以减轻认知功能障碍，且阻止进一步突触丧失、轴突变性和神经元细胞死亡。Aβ可以主动转运穿过血脑屏障(Deane等人，Stroke 2004;35(增刊I):2628-31)。在AD的鼠类模型中，针对Aβ的抗体的全身性递送增加血浆中的Aβ含量，同时借助若干提出机制来降低中枢神经系统(CNS)中的含量，包括大脑Aβ斑块的溶解、经调理Aβ的噬菌作用去除和最终由于循环抗体所致的Aβ平衡移位而使Aβ自大脑流出(Morgan,Neurodegener.Dis.2005;2:261-6)。

[0008] 重大失败记录了用于治疗AD的治疗抗体的发展。贝平珠单抗(bapineuzumab)(一种特异性结合至Aβ的N末端部分的抗体)的大规模3期临床试验因施用药物未能阻止所治疗患者的认知衰退而终止(Miles等人，Scientific Reports 2013;3:1-4Johnston&Johnson

press,2012年8月6日发布,标题为“Johnson&Johnson Announces Discontinuation of Phase 3Development of Bapineuzumab Intravenous(IV)in Mild-To-Moderate Alzheimer's Disease”)。显著地,贝平珠单抗似乎稳定斑块含量,且降低脑脊髓液中的磷酸化tau含量,这表明只有这些生物标记物的改善未必能预测临床效能(Miles等人,Scientific Reports 2013;3:1-4)。类似地,在苏兰珠单抗(solanezumab)(一种特异性针对单体Aβ的在肽的中间部分结合的抗体)的3期临床试验中,未满足主要认知和功能终点(Eli Lilly and Company press,2012年8月24日发布,“Eli Lilly and Company Aanounces Top-Line Results on Solanezumab Phase 3Clinical Trials in Patients with Alzheimer's Disease”)。在研究用于AD的某些免疫疗法期间,也发生了安全性问题;例如,在贝平珠单抗的2期临床试验中,在药物治疗的患者中,淀粉样蛋白相关的成像异常(ARIA-E和ARIA-H)的发生率超过20%(Sperling等人,The Lancet 2012;11:241-249)。据估计,在9个年龄超过65岁的人中就有一个患有AD,患有AD的个体和其代表用于健康照护、长期照护和临终关怀的每年总费用在2013年超过$2000亿,且估计2050年上升至$1.2万亿(患病个体和其代表)(Alzheimer's Association 2013Alzheimer's Disease Facts and Figures,Alzheimer's and Dementia 9:2)。截至2013年,AD在美国是第六大死亡原因(同上)。目前批准的疗法仅治疗AD的一些症状,而未能根本地消退。对于用于AD的疾病改善治疗存在巨大的未满足需求。

## 发明概要

[0009] 克雷内治单抗(Crenezumab,也称为MABT5102A)是Aβ的完全人源化的IgG4单克隆抗体,针对其在体外结合Aβ的单体和低聚物形式的能力进行筛选。克雷内治单抗结合Aβ1-40和Aβ1-42二者,抑制Aβ聚集,并且促进Aβ解聚。因为克雷内治单抗是人类IgG4骨架抗体,所以其相比于人类IgG1或IgG2具有降低的Fcγ受体(“FcγR”)结合亲和力,这可以预测减少的免疫效应反应。这些性质与全身递送的克雷内治单抗在AD的鼠科动物模型中降低AβCNS水平的能力相结合已经表明这种抗Aβ治疗方法可以提供临床疗效,同时降低毒性的风险,并且可能潜在地能够改变AD的疾病进展并降低潜在有害的副作用(诸如脑血管性水肿或出血)的风险,这些副作用之前已见于其它Aβ抗体疗法的临床试验中。

[0010] 本文所述的在AD患者中的二期临床研究的结果证实:克雷内治单抗的确减缓轻到中度AD的疾病进展,在ApoE4阳性患者和罹患轻度AD的患者中具有甚至更强的效果,并且在患有最轻度AD的患者中显示最大的治疗益处。此外,在具有通常见于被诊断为AD的患者中的脑淀粉样蛋白负荷的患者中观察到所述效果。另外,结果表明:这些效果是在无不良事件如ARIA-E和ARIA-H的显著发生率的情况下发生。因此,本申请提供用于治疗和监测被诊断为轻到中度AD(特别是轻度AD)的患者和ApoE4阳性患者,以及具有通常见于被诊断为AD的患者中的脑淀粉样蛋白累积的患者的方法。如本文所例示,现在已经发现:具有特异于淀粉样蛋白β(Aβ)肽的中间区域的构象表位(即,在氨基酸13-24内,如克雷内治单抗)的人源化单克隆抗淀粉样蛋白β抗体能有效治疗轻到中度AD,特别是ApoE4阳性患者和患有轻度形式的AD(诸如但不限于轻度AD)的患者,而不增加ARIA-E或ARIA-H的发生率。因此,本申请提供用于调节AD的严重度的治疗剂和使用它们的改良方法。

[0011] 因此,本申请提供治疗罹患AD和其它淀粉样变性的患者的方法,其包括施用结合

在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内的人源化单克隆抗淀粉样蛋白β(Aβ或Abeta)抗体或其抗原结合片段。在一些实施方案中,所述抗体或其抗原结合片段能够结合Aβ的原纤维、低聚物和单体形式。在一些实施方案中,抗体是IgG4抗体。在特定实施方案中,所述抗体或其抗原结合片段包含六个高变区(HVR),其中HVR-H1是SEQ ID NO:2,HVR-H2是SEQ ID NO:3,HVR-H3是SEQ ID NO:4,HVR-L1是SEQ ID NO:6,HVR-L2是SEQ ID NO:7,且HVR-L3是SEQ ID NO:8。在一些实施方案中,所述抗体或其抗原结合片段包含具有SEQ ID NO:5的氨基酸序列的重链(包含重链可变区),和具有SEQ ID NO:9的氨基酸序列的轻链(包含轻链可变区)。在一个具体实例中,所述抗体是克雷内治单抗。

[0012] 本文所提供的治疗方法可以应用于罹患AD或如本文进一步所述的其它淀粉样变性的患者。合适的患者包括罹患轻到中度AD的患者、具有18至26的MMSE评分的患者、罹患轻度AD的患者、具有20或以上(例如,20-30、20-26、24-30、21-26、22-26、22-28、23-26、24-26或25-26)的MMSE评分的患者、罹患早期AD的患者(包括具有由AD引起的轻度认知障碍的患者和具有临床前AD的患者)、淀粉样蛋白阳性患者(或具有与见于被诊断为AD的患者中的脑淀粉样蛋白负荷相一致的脑淀粉样蛋白负荷的患者)以及罹患轻到中度或轻度AD的ApoE4阳性患者。

[0013] 在一些方面,本文提供的方法是减少罹患早期、轻度或轻到中度AD的患者中由AD引起的衰退的方法。在一些实施方案中,衰退是下列中的一个或多个:临床衰退、认知衰退和功能衰退。在一些实施方案中,衰退是临床衰退。在一些实施方案中,衰退是认知能力的衰退或认知衰退。在一些实施方案中,衰退包括功能能力的衰退或功能衰退。已经开发出各种测试和量表来测量认知能力(包括记忆)和/或功能。在各种实施方案中,使用一种或多种测试来测量临床、功能或认知衰退。认知能力的标准测量是阿尔茨海默氏病评估量表认知(ADAS-Cog)测试,例如12项ADAS-Cog或ADAS-Cog12。因此,在一些实施方案中,使用ADAS-Cog12测试来确定经本发明抗体治疗的患者中的认知能力衰退(或认知衰退)的减少或减缓。ADAS-Cog12评分的增加表明患者病状的恶化。在一些实施方案中,通过临床痴呆评定量表/得分总和(CDR-SOB)评分来确定经本发明抗体治疗的患者中的认知衰退(或认知能力的衰退)的减少或减缓。在一些实施方案中,使用工具性日常生活活动(iADL)量表来确定经本发明抗体治疗的患者中的功能衰退(或功能能力的衰退)的减少或减缓。在一些实施方案中,评估一种或多种类型的衰退并使用前述测试或量表中的一个或多个来测量衰退的减少或减缓。

[0014] 本发明的抗体或其抗原结合片段是以有效治疗AD或如本文所述的其它淀粉样变性的剂量来施用。合适的剂量描述于本文中并且可以介于约0.3mg/kg至100mg/kg的范围内。在一个示例性实施方案中,剂量是15mg/kg。在另一个示例性实施方案中,剂量是30mg/kg。在另一个示例性实施方案中,剂量是45mg/kg。在一些实施方案中,剂量是在500mg与1000mg之间,例如500mg、700mg、720mg、750mg、800mg、820mg、900mg,或在1000mg与2500mg之间,例如1050mg、1500mg或2100mg。在本文所提供的方法中,预期了各种剂量方案,包括其中在长期的时间段内(例如几个月至几年)重复(例如每周或每月一次)施用所述抗体。

[0015] 本公开的人源化单克隆抗Aβ抗体提供的另一个益处在于其不增加不良事件如ARIA-E和ARIA-H的发生率。如本文中所示,相对于安慰剂组,治疗组中的这些不良事件没有增加。因此,本公开进一步提供治疗罹患轻到中度AD或轻度AD的患者而不增加不良事件如

ARIA-E和/或ARIA-H的发生率的方法。

[0016] 本公开进一步提供适合在本文所公开的治疗方法中使用的药物制剂。所述药物制剂可以被配制成用于任何方便的施用途径例如肠胃外或静脉注射，并且除本公开的抗Aβ以外通常将包括适于所需施用模式的一种或多种可接受的载剂、赋形剂和/或稀释剂。在一些实施方案中，本发明的抗体可以配制来用于静脉内施用。在一些实施方案中，可以在精氨酸缓冲液(例如，精氨酸琥珀酸盐缓冲液)中配制本发明的抗体。缓冲液可以包含一种或多种表面活性剂，例如聚山梨醇酯。在某些实施方案中，缓冲液浓度为50mM或更高。在一些实施方案中，pH在4.5和7.0之间，例如pH 5.5。本文描述了另外的实施方案。药物制剂可以包装在单位剂型中以便于使用。

[0017] 用于治疗AD或如本文所述的其它淀粉样变性的抗Aβ抗体治疗可以与其它疗法(包括除克雷内治单抗以外的一个或多个抗Aβ抗体)组合。其它疗法的非限制性实例包括神经药物、皮质类固醇、抗生素和抗病毒剂。除克雷内治单抗以外的抗Aβ抗体的非限制性实例包括苏兰珠单抗、贝平珠单抗、阿达鲁单抗(aducanumab)和格特鲁单抗(gantenerumab)。

[0018] 附图简述

[0019] 图1提供Aβ(1-42)(SEQ ID NO:1)的氨基酸序列，其中氨基酸13至24加有下划线。

[0020] 图2提供三个重链高变区(分别为HVR-H1、HVR-H2和HVR-H3)的氨基酸序列和三个轻链区(分别为HVR-L1、HVR-L2、HVR-L3)的氨基酸序列。

[0021] 图3提供克雷内治单抗的重链氨基酸序列(SEQ ID NO:5)，其包含跨越SEQ ID NO:5的氨基酸1至112的重链可变区；和轻链氨基酸序列(SEQ ID NO:9)，其包含跨越SEQ ID NO:9的氨基酸1至112的轻链可变区。SEQ ID NO:5和SEQ ID NO:9中的下划线示出了对应于SEQ ID NO:2-4的三个重链HVR的氨基酸序列和对应于SEQ ID NO:6-8的三个轻链HVR的氨基酸序列。

[0022] 图4A-B提供了招募在实施例1中所述的临床试验中的患者的概要，列出了每个组(治疗对安慰剂)中招募的患者的数量、ApoE4状态(ApoE4阴性/ApoE4阳性)、AD的阶段(轻度或中度)，以及筛选时的MMSE评分、针对AD症状的同步疗法(conmed使用)的存在和类型。

[0023] 图5提供了描述于实施例1中的临床试验的示意图，其示出了给药时间表、用量和途径。

[0024] 图6A-B提供了数据表，其示出了治疗组和安慰剂组中相对于基线的第73周ADAS-Cog12评分的变化。图6A提供了关于患有轻到中度AD、轻度AD、中度AD的患者以及ApoE4阳性和阴性患者的数据。图6B根据MMSE评分提供关于患者的数据。

[0025] 图7提供了针对用克雷内治单抗(深色实线)或安慰剂(浅色实线)治疗的患有轻度AD且MMSE评分在20和26之间的患者的ADAS-Cog12评分的变化的图表。

[0026] 图8提供了针对用克雷内治单抗(深色实线)或安慰剂(浅色实线)治疗的患有轻到中度AD且MMSE评分在18和26之间的患者的ADAS-Cog12评分的变化的图表。

[0027] 图9提供了针对用克雷内治单抗(深色实线)或安慰剂(浅色实线)治疗的患有轻到中度AD的ApoE4阳性患者的ADAS-Cog12评分的变化的图表。

[0028] 图10提供了用克雷内治单抗(深色实线)或安慰剂(浅色实线)治疗的所有ApoE4阳性患者和患有轻度AD的患者的ADAS-Cog12评分的变化的图表。

[0029] 图11提供了针对用克雷内治单抗或安慰剂治疗的患有轻度AD且MMSE评分在22和

26之间的患者的ADAS-Cog12评分的变化的图表。

[0030] 图12A-B提供了数据表，其示出了治疗组和安慰剂组中相对于基线的第73周CDR-SOB评分的变化。图12A根据MMSE评分提供关于患者的CDR-SOB评分的变化的数据。图12B提供关于MMSE评分在18-26、20-26和22-26的范围内的患者的CDR-SOB评分以及CDR判断和问题解决评分和CDR记忆评分的数据。

[0031] 图13提供了针对用克雷内治单抗或指定安慰剂治疗的患有轻度AD且MMSE评分为25或26的患者的CDR-SOB评分的变化的图表。

[0032] 图14A-B提供了招募在实施例2中所述的临床试验中的患者在基线处和治疗后的概要，包括不良事件数据(A)和显示何时在临床试验中进行PET扫描、MRI扫描和CSF抽样的时间轴(B)。

[0033] 图15A-B提供了显示接受安慰剂(虚线)或克雷内治单抗(实线)的患者中的淀粉样蛋白水平的图表，如在接受安慰剂或克雷内治单抗的患者中通过PET分析进行的florbetapir成像(A)和CSF Aβ水平(B)所测定。

## 具体实施方式

[0034] 除非另有规定，否则本文所使用的技术和科技术语具有如本发明所属技术领域的一般技术人员通常所理解的相同含义。Singleton等人，Dictionary of Microbiology and Molecular Biology第2版，J.Wiley&Sons(New York,N.Y.1994)和March,Advanced Organic Chemistry Reactions,Mechanisms and Structure第4版，John Wiley&Sons(New York,N.Y.1992)向本领域技术人员提供关于在本申请中使用的许多术语的一般指导。

[0035] 某些定义和缩写

[0036] 为了解释本说明书的目的，以下定义将适用，并且在任何适当的时候，以单数形式使用的术语还将包括复数，且反之亦然。如果下文阐述的任何定义与通过引用并入本文的任何文件有冲突，则以下文阐述的定义为准。

[0037] 如在本说明书和随附权利要求书中所使用，单数形式“一”、“一个”和“该”包括复数的指示对象，除非上下文另有明确说明。因此，例如，提及“一种蛋白质”或一种“抗体”分别包括多种蛋白质或抗体；提及“一个细胞”包括细胞的混合物等等。

[0038] 在本说明书和所附权利要求书中提供的范围包括两个端点和端点之间的所有点。因此，例如，2.0到3.0的范围包括2.0、3.0和2.0与3.0之间的所有点。

[0039] 如本文所使用，短语“大体上类似的”或“大体上相同的”表示两个数值之间具有足够高的相似程度(通常一个与本发明的抗体相关，且另一个与参照/比较抗体相关)，从而使得本领域技术人员将认为这两个值之间的差异在由所述值(例如，Kd值)衡量的生物特性的情况下很少有或没有生物学和/或统计显著性。所述两个值之间的差异作为参照/比较抗体的值的函数小于约50％、小于约40％、小于约30％、小于约20％、小于约10％。

[0040] 如本文中所使用，术语“样品”或“测试样品”是指从目标受试者获得或来源于目标受试者的组合物，其含有将要例如基于物理、生化、化学和/或生理特性来表征和/或鉴别的细胞和/或其它分子实体。在一个实施方案中，该定义包括血液和生物起源的其它液体样品以及组织样品诸如活检样本或组织培养物或由其衍生的细胞。组织样品的来源可以是来自新鲜、冷冻和/或保存的器官或组织样品或活组织切片或抽吸物的实体组织；血液或任何血

液成分;体液;以及来自受试者的妊娠或发育中的任何时间的细胞或血浆。如本文所使用,术语“生物样品”包括但不限于血液、血清、血浆、痰、组织活检物(例如,肺样品)和鼻腔样品,包括鼻拭物或鼻息肉。

[0041] 术语“样品”、“生物样品”或“测试样品”包括已在其获得之后以任何方式被操纵的生物样品,所述操纵是诸如通过用试剂处理、增溶或富集某些组分如蛋白质或多核苷酸或嵌入用于切片目的的半固体或固体基质中。为了本文中的目的,组织样品的“切片”意指组织样品的单个部分或一块,例如组织薄切片或从组织样品切除的细胞。样品包括但不限于全血、血液源性细胞、血清、血浆、淋巴液、滑液、细胞提取物和其组合。在一个实施方案中,样品为临床样品。在另一个实施方案中,样品被用于诊断分析。

[0042] 在一个实施方案中,样品是从经过抗Aβ抗体治疗之前的受试者或患者获得。在另一个实施方案中,样品是从经过抗Aβ抗体的至少一种治疗后的受试者或患者获得。

[0043] 如本文中所使用,“参照样品”是指用于比较目的的任何样品、标准或水平。在一个实施方案中,参照样品是从相同受试者或患者的身体的健康和/或未患病部分(例如,组织或细胞)获得。在另一个实施方案中,参照样品是从相同受试者或患者的身体的未经处理的组织或细胞获得。在又一个实施方案中,参照样品是从非受试者或患者的个体的身体的健康和/或未患病部分(例如,组织或细胞)获得。在甚至另一个实施方案中,参照样品是从非受试者或患者的个体的身体的未经处理的组织或细胞部分获得。

[0044] 在某些实施方案中,参照样品是在不同于获得测试样品时的一个或多个时间点从相同的受试者或患者获得的单个样品或合并的多个样品。例如,参照样品是在早于获得测试样品时的时间点从相同的受试者或患者获得。在某些实施方案中,参照样品包括如在术语“样品”下所定义的从一个或多个非受试者或患者的个体获得的所有类型的生物样品。在某些实施方案中,参照样品是从患有淀粉样变性(例如,阿尔茨海默氏病)的一个或多个非受试者或患者的个体获得。

[0045] 在某些实施方案中,参照样品是来自一个或多个非受试者或患者的健康个体的多个样品的组合。在某些实施方案中,参照样品是来自患有疾病或病症(例如,淀粉样变性如(例如)阿尔茨海默氏病)的一个或多个非受试者或患者的个体的合并的多个样品。在某些实施方案中,参照样品是来自正常组织的汇集RNA样品或来自一个或多个非受试者或患者的个体的汇集血浆或血清样品。

[0046] 术语“小分子”是指分子量在50道尔顿至2500道尔顿之间的有机分子。

[0047] 术语“抗体”和“免疫球蛋白”(“Ig”)在广义上可互换使用,且包括但不限于单克隆抗体(例如,全长或完整单克隆抗体)、多克隆抗体、多价抗体、具有多表位特异性的抗体、单链抗体、多特异性抗体(例如,双特异性抗体、三特异性抗体、四特异性抗体)和抗体片段,只要它们展现出期望的生物活性即可。此类抗体可以是嵌合的、人源化的、人的、合成的和/或亲和力成熟的。本文更详细地描述了此类抗体和生成它们的方法。

[0048] “抗体片段”只包含完整抗体的一部分,其中所述部分优选保留当该部分存在于完整抗体中时通常与其相关的功能中的至少一种且通常为大多数或全部。在一个实施方案中,抗体片段包含完整抗体的抗原结合位点且因此保留结合抗原的能力。在另一个实施方案中,抗体片段(例如包含Fc区的抗体片段)保留当该Fc区存在于完整抗体中时通常与其相关的生物功能中的至少一种,诸如FcRn结合、抗体半衰期调节、ADCC功能和补体结合。在一

个实施方案中，抗体片段是具有大体上类似于完整抗体的体内半衰期的单价抗体。例如，这样的抗体片段可以包含连接至能够赋予该片段体内稳定性的Fc序列的抗原结合臂。抗体片段的实例包括但不限于Fv、Fab、Fab'、Fab'-SH、F(ab')2；双抗体；线性抗体；单链抗体分子(例如scFv)；和由抗体片段形成的多特异性抗体。

[0049] 除非另外说明，否则本文所使用的术语“标靶”是指来自任何脊椎动物来源的任何天然分子，脊椎动物来源包括哺乳动物，例如灵长类动物(例如，人类)和啮齿动物(例如，小鼠和大鼠)。该术语包括“全长”未加工的标靶以及通过在细胞中处理所得到的任何形式的标靶。该术语还包括标靶的天然存在的变体，例如剪接变体或等位基因变体。

[0050] 在本文中可互换使用的术语“淀粉样蛋白β”、“β-淀粉样蛋白”、“Abeta”、“淀粉样蛋白β”和“Aβ”是指在淀粉样前体蛋白(“APP”)被β-分泌酶1(“BACE1”)裂解时产生的APP的片段，以及其修饰物、片段和任何功能等效物，包括但不限于Aβ1-40和Aβ1-42。已知Aβ以单体形式存在，也缔合形成低聚物和原纤维结构，其可以作为淀粉样蛋白斑的组成成员被发现。此类Aβ肽的结构和序列是本领域普通技术人员所熟知的，并且产生所述肽或从脑和其它组织中提取它们的方法描述于例如Glenner和Wong，Biochem Biophys Res.Comm.129:885-890(1984)。此外，Aβ肽还可以以各种形式购得。人类Aβ1-42的示例性氨基酸序列为DAEFRHDSGYEVHHQKLVFFAED VGSNKGAIIGLMVGGVVIA(SEQ ID NO:1)。

[0051] 术语“抗标靶抗体”和“结合标靶的抗体”是指这样的抗体，其能够以足够的亲和力结合标靶，从而使得该抗体可以作为诊断剂和/或治疗剂用于靶向标靶。在一个实施方案中，抗标靶抗体与无关的非标靶蛋白质的结合程度小于该抗体与标靶的结合的约10％，如例如通过放射免疫分析(RIA)或biacore分析所测量。在某些实施方案中，结合标靶的抗体具有≤1μM、≤100nM、≤10nM、≤1nM、≤0.1nM、≤0.01nM或≤0.001nM(例如，$10^{-8}$M或更小，例如$10^{-8}$M至$10^{-13}$M，例如$10^{-9}$M至$10^{-13}$M)的解离常数(Kd)。在某些实施方案中，抗标靶抗体与标靶的表位结合，该表位在不同物种之间是保守的。

[0052] “抗Aβ免疫球蛋白”、“抗Aβ抗体”和“结合Aβ的抗体”在本文中可互换使用，并且是指与人类Aβ特异性结合的抗体。抗Aβ抗体的非限制性实例是克雷内治单抗。抗Aβ抗体的其它非限制性实例为苏兰珠单抗、贝平珠单抗、阿达鲁单抗和格特鲁单抗。

[0053] 术语“克雷内治单抗”和“MABT5102A”在本文中可互换使用，并且是指结合Aβ的单体、低聚物和原纤维形式的特异性抗Aβ抗体，其与CAS登记号1095207相关联。在一个实施方案中，所述抗体包含图2中列出的HVR区序列。在另一个这样的实施方案中，所述抗体包含：(1)含有氨基酸序列SEQ ID NO:2的HVR-H1；(2)含有氨基酸序列SEQ ID NO:3的HVR-H2序列；(3)含有氨基酸序列SEQ ID NO:4的HVR-H3序列；(4)含有氨基酸序列SEQ ID NO:6的HVR-L1序列；(5)含有氨基酸序列SEQ ID NO:7的HVR-L2序列；和(6)含有氨基酸序列SEQ ID NO:8的HVR-L3序列。在另一个实施方案中，特异性抗Aβ抗体包含具有图3中列出的氨基酸序列的VH和VL结构域。在另一个这样的实施方案中，所述特异性抗Aβ抗体包含含有氨基酸序列SEQ ID NO:5的VH结构域和含有氨基酸序列SEQ ID NO:9的VL结构域。在另一个实施方案中，抗体是IgG4抗体。在另一个这样的实施方案中，IgG4抗体包含在其恒定结构域中的突变，使得丝氨酸228被脯氨酸替代。

[0054] 如本文中所使用，术语“淀粉样变性”是指由淀粉样蛋白或淀粉样蛋白样蛋白引起或与其相关的一组疾病和病症，并且包括但不限于由单体、原纤维或聚合物状态的淀粉样

蛋白样蛋白或所述三种的任意组合(包括淀粉样蛋白斑)的存在或活性引起的疾病和病症。此类疾病包括但不限于继发性淀粉样变性和年龄相关性淀粉样变性,诸如包括但不限于下述的疾病:神经系统病症如阿尔茨海默氏病(“AD”)、以先天性记忆能力丧失为特征的疾病或病状如(例如)轻度认知障碍(MCI)、路易体痴呆症(Lewy body dementia)、唐氏综合症(Down's syndrome)、遗传性脑出血伴淀粉样变性(Dutch型)、关岛帕金森病-痴呆复合征(Guam Parkinson-Demential complex)和其它基于淀粉样蛋白样蛋白或与其相关的疾病,诸如进行性核上麻痹、多发性硬化、克雅病(Creutzfeld Jacob disease)、帕金森病(Parkinson's disease)、HIV相关性痴呆、ALS(肌萎缩性侧索硬化症)、包含体肌炎(IBM)、成年发作型糖尿病、内分泌肿瘤和老年心脏淀粉样变性,以及多种眼部疾病,包括黄斑变性、玻璃疣相关性视神经病变、青光眼和由β-淀粉样蛋白沉积引起的白内障。

[0055] 青光眼为涉及视网膜神经节细胞(RGC)损失的一组视神经疾病,它们是视神经病变的特征模式。RGC是将视觉信号从眼睛传输至脑部的神经细胞。半胱天冬酶-3和半胱天冬酶-8是细胞凋亡过程中的两种主要的酶,它们在导致RGC凋亡的过程中被激活。半胱天冬酶-3使淀粉样前体蛋白(APP)裂解产生神经毒性片段,包括Aβ。没有APP的保护作用,Aβ在视网膜神经节细胞层中累积,导致RGC的死亡和不可逆的视力损失。

[0056] 青光眼通常(但不总是)伴有眼压增高,这可能是由于液体循环或其引流的阻塞而导致的。尽管眼内压升高是发展为青光眼的重要风险因素,但是无法限定导致青光眼的决定性眼内压阈值。损害还可能由于对重要视神经纤维的不良血液供给、神经结构的脆弱和/或神经纤维自身的健康问题而引起。未治疗的青光眼造成视神经的永久损害和随后的视野损失,这可能发展为失明。

[0057] 不同类型的青光眼可以分类为开角型青光眼(如果病状是慢性的)或闭角型青光眼(如果急性青光眼突然发生)。青光眼通常影响两只眼睛,但是疾病在一只眼睛中较在另一只眼睛中的进展可能更迅速。

[0058] 慢性开角型青光眼(COAG)也称为原发性开角型青光眼(POAG),它是最常见类型的青光眼。COAG是由小梁网中的微小阻塞而引起,它减少液体外流到输淋氏管(Schlemm's canal)中的引流并提高眼内压(IOP)。POAG通常影响两只眼睛,并且与年龄以及阳性家族史密切相关。其发生率在老年人群中增加,因为眼部引流机制可能随着年龄增加而逐渐阻塞。受慢性开角型青光眼影响的受试者中眼内压增加不伴发任何症状,直到感觉到中枢视觉区域的损失。

[0059] 急性闭角青光眼(AACG)或闭角型青光眼是相对罕见类型的青光眼,其特征在于眼内压突然增加至35至80mmHg,导致严重疼痛和不可逆的视力损失。压力的突然增加是由于过滤角的闭合和引流管的阻塞而引起。具有窄角的个体具有增加的角突然闭合的风险。AACG通常在一只眼睛中发生,但是风险存在于两只眼中。年龄、白内障和假性剥脱综合症也是风险因素,因为它们与晶状体的增大以及角的挤塞或狭窄有关。青光眼的突然发作可以与严重的眼痛和头痛、眼部发炎、恶心、呕吐以及视力模糊相关。

[0060] 混合或合并机制青光眼为开角型和闭角型青光眼的混合体或组合体。它影响急性ACG患者(在激光虹膜切开术后角开放,但是继续需要控制IOP的药物治疗)以及POAG患者或假性剥脱性青光眼患者,后者逐渐发展为角狭窄。

[0061] 正常眼压性青光眼(NTG)也称为低眼压性青光眼(LTG),其特征在于渐进性视神经

损害和类似于在其它类型青光眼中所见的周边视力丧失;然而,眼内压在正常范围内,或者甚至低于正常范围。

[0062] 先天性(婴儿期)青光眼是相对罕见的遗传类型的开角型青光眼。引流区域的发育不足导致眼压增加,这会导致由于视神经损害而引起的视力丧失和眼睛变大。早期诊断和治疗对于受疾病影响的婴儿和儿童的视力保护是至关重要的。

[0063] 继发性青光眼可能是由于眼损伤、眼虹膜炎症(虹膜炎)、糖尿病、白内障或在类固醇敏感性个体中使用类固醇而引起。继发性青光眼还可能与视网膜脱落或视网膜静脉阻塞或堵塞有关。

[0064] 色素性青光眼的特征在于色素颗粒从虹膜脱离。所述颗粒导致眼睛的引流系统的阻塞,使得眼内压升高并损害视神经。剥脱性青光眼(假性剥脱综合症)的特征在于薄片状物在前囊上和眼角中的沉积。薄片状物的累积堵塞引流系统并使得眼压升高。

[0065] 青光眼的诊断可以使用各种测试进行。眼压测量法通过测定眼表面的紧张性或硬度来确定眼内的压力。多种类型的眼压计可以用于该测试,最常见的是压平眼压计。角膜测厚法测定角膜的厚度,其进而衡量眼内压。前房角镜检查法允许检查过滤角和眼睛的引流区域。前房角镜检查法还可以确定异常血管是否可能阻断水性流体流出眼睛。眼膜曲率镜法允许检查视神经,并且可以检测神经纤维层下降或视盘改变或该结构的压痕(杯状凹陷),这可能是由于眼内压升高或轴突掉出而导致。前房角镜检查法还可以用于评估由于血流不良或眼内压升高而导致的神经损害。视野测试主观地绘制视野图,它可以检测青光眼对视神经的损害的征象。这是由视野损失的特定模式来代表。光学相干断层扫描(神经纤维层损失的客观测量)是通过经由光透射通过损害的轴突组织的差异观察视神经纤维层的厚度(在青光眼中有改变)来进行。

[0066] 作为参考抗体的“结合相同表位的抗体”是指在竞争分析中阻断参考抗体与其抗原的结合达50%或更多的抗体,且相反地,该参考抗体在竞争分析中阻断抗体与其抗原的结合达50%或更多。本文提供了示例性竞争分析。

[0067] 术语“治疗剂”是指用于治疗疾病的任何药剂,包括但不限于治疗疾病的症状的药剂。

[0068] 如本文所使用,“治疗”(和其语法变型,treatment/treat/treating)是指试图改变所治疗的个体中的自然过程的临床干预,并且可以在临床病理学的过程中进行。期望的治疗效果包括但不限于减轻或改善一种或多种症状、减少或延迟疾病的任何直接或间接病理结果的出现或恶化、降低疾病进展的速率以及改善或缓和疾病状态。在一些实施方案中,抗体被用于延缓疾病的发展或减缓疾病的进展。

[0069] 如本文所使用,术语“治疗出现的”是指在施用第一剂量的治疗剂后发生的事件。例如,“治疗出现的不良事件”是在临床研究中的第一剂量的治疗之时或之后确定的事件。

[0070] “治疗方案”是指剂量、施用频率或治疗持续时间与添加或不添加第二药物的组合。

[0071] “有效治疗方案”是指将对接受治疗的患者提供有益反应的治疗方案。

[0072] “改变治疗”是指改变治疗方案,包括改变剂量、施用频率或治疗持续时间,和/或添加第二药物。

[0073] 药剂的“有效量”或“有效剂量”是指在必要的时间段内能有效实现所需结果的量

或剂量。例如，“治疗有效量”是在必要的时间段内能有效治疗所指示的疾病、病状、临床病理或症状(即，更改AD的进程和/或减轻和/或预防AD的一种或多种症状)的量。

[0074] “亲和力”或“结合亲和力”是指分子(例如抗体)的单一结合位点与其结合配偶体(例如抗原)之间的非共价相互作用总和的强度。除非另外指示，否则如本文所使用，“结合亲和力”是指反映结合对的成员(例如抗体和抗原结合臂)之间的1:1相互作用的内在结合亲和力。分子X对其配偶体Y的亲和力通常可以由解离常数(Kd)表示。亲和力可以通过本领域中已知的普通方法来测量，包括本文中描述的那些方法，其中任何一种都可以用于本发明的目的。关于测量结合亲和力的具体的说明性和示例性实施方案描述于本文中。

[0075] “亲和力成熟的”抗体是指在一个或多个高变区(HVR)中具有一个或多个改变的抗体，与不具有这些改变的亲本抗体相比，这些改变改善抗体对抗原的亲和力。

[0076] 如本文中所使用，术语“患者”是指需要进行治疗的任何单个受试者。在某些实施方案中，本文中的患者是人类。

[0077] 本文中的“受试者”通常是人类。在某些实施方案中，受试者是非人类哺乳动物。示例性的非人类哺乳动物包括实验室、家养、宠物、运动和家畜动物，例如小鼠、猫、狗、马和牛。通常，受试者适于接受治疗，例如，展现疾病的一种或多种标记。通常，这样的受试者或患者适于接受针对淀粉样变性例如AD的治疗。在一个实施方案中，这样的合格受试者或患者是正在经历或已经历AD的一种或多种征象、症状或其它指标或已被诊断为患有AD(无论是例如初诊、先前诊断或有发展为AD的风险)的受试者或患者。AD的诊断可以基于临床病史、临床检查和确立的成像模式来进行。本文中的“患者”或“受试者”包括适于接受治疗的任何单个人类受试者，其正在经历或已经历AD的一种或多种征象、症状或其它指标。意欲被包括来作为受试者的是参与临床研究试验的任何受试者，或参与流行病学研究的受试者，或曾经被用作对照的受试者。受试者可能早先已经用抗Aβ抗体或其抗原结合片段或另一种药物治疗过，或没有经过这样的治疗。受试者在本文中的治疗开始时可以未经过额外药物的治疗，即，受试者可能早先没有用例如除抗Aβ以外的疗法在“基线”处(即，在本文的治疗方法中施用第一剂量的抗Aβ之前的设定时间点，诸如在治疗开始前筛选受试者的当天)进行治疗。此类“未经过治疗的”受试者通常被认为是使用此类额外药物进行治疗的候选者。

[0078] 如本文所使用，受试者的“寿命”是指受试者在开始治疗后的生命的剩余部分。

[0079] 如本文所使用，术语“单克隆抗体”是指从大体上同质性抗体的群体(即，除了可以少量存在的可能的天然存在的突变外，构成该群体的个别抗体是相同的)获得的抗体。单克隆抗体是高度特异性的，针对单一抗原。此外，与通常包括针对不同决定簇(表位)的不同抗体的多克隆抗体制剂相反，每个单克隆抗体是针对抗原上的单一决定簇。

[0080] 单克隆抗体在本文中具体包括“嵌合”抗体，其中重链和/或轻链的一部分与衍生自特定物种或属于特定抗体类别或亚类的抗体中的相应序列相同或同源，而所述链的剩余部分与衍生自另一物种或属于另一抗体类别或亚类的抗体中的相应序列相同或同源；以及此类抗体的片段，只要它们展现出期望的生物活性(美国专利号4,816,567；和Morrison等人，Proc.Natl.Acad.Sci.USA 81:6851-6855(1984))。

[0081] 抗体的“类别”是指其重链所具有的恒定结构域或恒定区的类型。有五种主要的抗体类别：IgA、IgD、IgE、IgG和IgM，并且这些中的若干个可以被进一步分成亚类(或同种型)，例如IgG1、IgG2、IgG3、IgG4、IgA1和IgA2。对应于不同免疫球蛋白类别的重链恒定结构域分

别被称为、、、和。

[0082] 非人类(例如鼠科动物)抗体的“人源化”形式是包含来源于非人类免疫球蛋白的最小序列的嵌合抗体。对于主要部分,人源化抗体是人类免疫球蛋白(受体抗体),其中该受体的高变区的残基被具有所需特异性、亲和力和能力的非人类物种(供体抗体)(诸如小鼠、大鼠、兔或非人类灵长类动物)的高变区的残基替换。在一些情形中,人类免疫球蛋白的框架区(FR)残基被相应的非人类残基替换。此外,人源化抗体可以包含在受体抗体或在供体抗体中不存在的残基。进行这些修饰以进一步改善抗体性能。通常,人源化抗体将包含大体上所有至少一个和通常两个可变结构域,其中所有或大体上所有高变环对应于非人类免疫球蛋白的高变环,且所有或大体上所有FR是人类免疫球蛋白lo序列的FR。人源化抗体任选地还将包含免疫球蛋白恒定区(Fc)(通常是人类免疫球蛋白的恒定区)的至少一部分。更多细节参见Jones等人,Nature 321:522-525(1986);Riechmann等人,Nature 332:323-329(1988);和Presta,Curr.Op.Struct.Biol.2:593-596(1992)。还参见以下综述文章和其中引用的参考文献:Vaswani和Hamilton,Ann.Allergy,Asthma&Immunol.1:105-115(1998);Harris,Biochem.Soc.Transactions 23:1035-1038(1995);Hurle和Gross,Curr.Op.Biotech.5:428-433(1994)。

[0083] “人类抗体”是包含与由人类或人类细胞生成的抗体相对应的氨基酸序列和/或已衍生自利用人类抗体全集或其它人类抗体编码序列的非人类来源的抗体,所述人类抗体是例如使用如本文所公开的任何制备人类抗体的技术而制得。此类技术包括但不限于:筛选人源性组合文库,诸如噬菌体展示文库(参见例如Marks等人,J.Mol.Biol.,222:581-597(1991)和Hoogenboom等人,Nucl.Acids Res.,19:4133-4137(1991));使用用于生成人类单克隆抗体的人骨髓瘤和小鼠-人杂交骨髓瘤细胞系(参见例如,Kozbor J.Immunol.,133:3001(1984);Brodeur等人,Monoclonal Antibody Production Techniques and Applications,第55-93页(Marcel Dekker,Inc.,New York,1987);和Boerner等人,J.Immunol.,147:86(1991));以及在能够在不产生内源性免疫球蛋白的情况下产生人类抗体的完整全集的转基因动物(例如小鼠)中生成单克隆抗体(参见例如,Jakobovits等人,Proc.Natl.Acad.Sci USA,90:2551(1993);Jakobovits等人,Nature,362:255(1993);Bruggermann等人,Year in Immunol.,7:33(1993))。人类抗体的这种定义明确排除含有来自非人类动物的抗原结合残基的人源化抗体。

[0084] “分离的”抗体是已经从其天然环境的组分中鉴别和分离和/或回收的抗体。其天然环境的污染物组分是将干扰抗体的诊断或治疗用途的物质,并且可以包括酶、激素和其它蛋白质或非蛋白质溶质。在一些实施方案中,抗体被纯化至大于95%或99%的纯度,如通过例如电泳法(例如SDS-PAGE、等电聚焦(IEF)、毛细管电泳)或色谱法(例如离子交换或逆相HPLC)所测定。关于抗体纯度的评估方法的综述,参见例如Flatman等人,J.Chromatogr.B 848:79-87(2007)。

[0085] 术语“可变区”或“可变结构域”是指抗体重链或轻链的涉及抗体与抗原的结合的结构域。天然抗体的重链和轻链的可变结构域(分别为VH和VL)通常具有类似的结构,其中每个结构域包含四个保守框架区(FR)和三个高变区(HVR)。(参见例如,Kindt等人Kuby Immunology,第6版,W.H.Freeman and Co.,第91页(2007)。)单个VH或VL结构域可以足以产生抗原结合特异性。此外,使用来自结合特定抗原的抗体的VH或VL结构域以分别筛选互补

的VL或VH结构域文库可以分离结合该抗原的抗体。参见例如Portolano等人，J.Immunol.150:880-887(1993);Clarkson等人,Nature 352:624-628(1991)。

[0086] 术语“高变区”、“HVR”或“HV”当在本文中使用时是指抗体可变结构域的在序列上高变和/或形成结构定义环的区域。通常,抗体包含六个高变区;三个在VH中(H1、H2、H3),且三个在VL中(L1、L2、L3)。许多高变区描述是在用的并且包括在本文中。Kabat互补决定区(CDR)是基于序列变异性并且是最常用的(Kabat等人,Sequences of Proteins of Immunological Interest,第5版,Public Health Service,National Institutes of Health,Bethesda,Md.(1991))。而Chothia是指结构环的位置(Chothia和Lesk J.Mol.Biol.196:901-917(1987))。AbM高变区代表Kabat CDR与Chothia结构环之间的折衷,并且被Oxford Molecular's AbM抗体建模软件使用。“接触”高变区是基于可用的复合晶体结构的分析。来自这些HVR中的每个的残基注释如下。

[0087]

| 环 | Kabat | AbM | Chothia | 接触 |
|---|---|---|---|---|
| L1 | L24-L34 | L24-L34 | L26-L32 | L30-L36 |
| L2 | L50-L56 | L50-L56 | L50-L52 | L46-L55 |
| L3 | L89-L97 | L89-L97 | L91-L96 | L89-L96 |
| H1 | H31-H35B | H26-H35B | H26-H32 | H30-H35B (Kabat编号) |
| H1 | H31-H35 | H26-H35 | H26-H32 | H30-H35 (Chothia编号) |
| H2 | H50-H65 | H50-H58 | H53-H55 | H47-H58 |
| H3 | H95-H102 | H95-H102 | H96-H101 | H93-H101 |

[0088] 高变区可以包含如下的“延伸高变区”:VL中的24-36或24-34(L1)、46-56或49-56或50-56或52-56(L2)和89-97(L3)以及VH中的26-35(H1)、50-65或49-65(H2)和93-102、94-102或95-102(H3)。可变结构域残基是按照Kabat等人(同上)针对这些定义中的每一个进行编号。

[0089] “框架”或“FR”残基是除如本文所定义的高变区残基以外的那些可变结构域残基。可变结构域的FR一般由四个FR结构域组成:FR1、FR2、FR3和FR4。因此,HVR和FR序列一般按以下顺序出现在VH(或VL)中:FR1-H1(L1)-FR2-H2(L2)-FR3-H3(L3)-FR4。

[0090] “受体人类框架”就本文的目的来说是包含源自如下所定义的人类免疫球蛋白框架或人类共有框架的轻链可变结构域(VL)框架或重链可变结构域(VH)框架的氨基酸序列的框架。“衍生自”人类免疫球蛋白框架或人类共有框架的受体人类框架可以包含其相同的氨基酸序列,或其可以包含氨基酸序列变化。在一些实施方案中,氨基酸变化的数量为10个或更少、9个或更少、8个或更少、7个或更少、6个或更少、5个或更少、4个或更少、3个或更少或2个或更少。在一些实施方案中,VL受体人类框架的序列与VL人类免疫球蛋白框架序列或人类共有框架序列相同。

[0091] “人类共有框架”是代表在人类免疫球蛋白VL或VH框架序列的选择中最常出现的氨基酸残基的框架。一般来说,人类免疫球蛋白VL或VH序列的选择来自可变结构域序列的子组。一般来说,序列的子组是如Kabat等人,Sequences of Proteins of Immunological Interest,第5版,NIH Publication 91-3242,Bethesda MD(1991),第1-3卷中的子组。等等。

[0092] 术语“淀粉样蛋白相关成像异常-水肿”或“ARIA-E”包括脑血管性水肿和脑沟积液。

[0093] 术语“淀粉样蛋白相关成像异常-出血”或“ARIA-H”包括微出血和中枢神经系统的表面铁沉积症。

[0094] 在本文中可与“载脂蛋白Ｅ4阳性”或“ApoE4阳性”互换使用的“载脂蛋白Ｅ4载体”或“ApoE4载体”是指具有至少一个载脂蛋白Ｅ4(或“ApoE4”)等位基因的个体。具有零个ApoE4等位基因的个体在本文中被称为“ApoE4阴性”或“ApoE4非载体”。还参见Prekumar等人,1996,Am.J Pathol.148:2083-95。

[0095] 术语“脑血管性水肿”是指血管内流体或蛋白质在大脑的细胞内或细胞外空间中的过量累积。脑血管性水肿可以通过例如脑MRI(包括但不限于FLAIR MRI)进行检测,并且可以是无症状的(“无症状性血管性水肿”)或与诸如意识错乱、头晕、呕吐和昏睡的神经症状相关(“症状性血管性水肿”)(参见Sperling等人Alzheimer’s&Dementia,7:367,2011)。

[0096] 术语“大脑大出血”是指直径大于约1cm的区域的颅内出血或脑中流血。大脑大出血可以通过例如脑MRI(包括但不限于T2*加权GRE MRI)进行检测,并且可以是无症状的(“无症状性大出血”)或与诸如暂时性或永久性局灶性运动障碍或感觉障碍、共济失调、失语症和构音困难的症状相关(“症状性大出血”)(参见例如Chalela JA,Gomes J.Expert Rev.Neurother.2004 4:267,2004和Sperling等人Alzheimer’s&Dementia,7:367,2011)。

[0097] 术语“大脑微出血”是指直径小于约1cm的区域的颅内出血或脑中流血。大脑微出血可以通过例如脑MRI(包括但不限于T2*加权GRE MRI)进行检测,并且可以是无症状的(“无症状性微出血”)或可以与诸如暂时性或永久性局灶性运动障碍或感觉障碍、共济失调、失语症和构音困难的症状相关(“症状性微出血”)。参见例如Greenberg等人,2009,Lancet Neurol.8:165-74。

[0098] 术语“脑沟积液”是指在大脑的脑沟或脑槽中流出的流体。脑沟积液可以通过例如脑MRI(包括但不限于FLAIR MRI)来检测。参见Sperling等人Alzheimer’s&Dementia,7:367,2011。

[0099] 术语“中枢神经系统表面铁沉积症”是指进入脑的蛛网膜下腔的流血或出血,并且可以通过例如脑MRI(包括但不限于T2*加权GRE MRI)进行检测。指示中枢神经系统表面铁沉积症的症状包括感觉神经性耳聋、小脑共济失调和锥体束征。参见Kumara-N,Am J Neuroradiol.31:5,2010。

[0100] 如本文中所使用,术语“进展”是指疾病随时间的恶化。疾病的“进展速率”或“进展的速率”是指疾病在被诊断为患有该疾病的患者中随时间发展的快慢。疾病的进展速率可以通过疾病的特定特征随时间的可测量变化来表示。如果携带特定遗传性状的患者的疾病状态的进展速度比没有这种遗传性状的那些患者快,则她被称为具有或更有可能具有“增加的进展速率”。在另一方面,响应于治疗的患者被称为具有或更有可能具有“降低的进展速率”,如果当与她在治疗前的疾病状态或与没有治疗的其它患者相比时,她的疾病进展在治疗后减缓。

[0101] 如本文所使用,“更有可能响应”是指最有可能展现出淀粉样变性(例如AD)的进展的减缓或预防的患者。就AD而言,“更有可能响应”是指最有可能展现出治疗减少功能或认知损失的患者。短语“响应于”在本发明的上下文中指示罹患、被怀疑罹患或容易罹患或被

诊断为患有如本文所述的病症的患者显示对抗Aβ治疗的反应。

[0102] 如本文中所使用,短语“选择患者”或“鉴别患者”是指使用所生成的涉及患者的样品中等位基因的存在的信息或数据来鉴别或选择更有可能受益于包含抗Aβ抗体的治疗的患者。所使用或生成的信息或数据可以为任何形式,书面、口头或电子的。在一些实施方案中,使用所生成的信息或数据包括传达、呈递、报告、存储、发送、传递、提供、传输、分配或其组合。在一些实施方案中,传达、呈递、报告、存储、发送、传递、提供、传输、分配或其组合是由计算设备、分析器单元或其组合来执行。在一些其它实施方案中,传达、呈递、报告、存储、发送、传递、提供、传输、分配或其组合是由实验室或医学专业人员来执行。在一些实施方案中,所述信息或数据包括特定等位基因存在或不存在于样品中的指示。在一些实施方案中,所述信息或数据包括患者更有可能对包含抗Aβ的疗法作出响应的指示。

[0103] “效应子功能”是指可归因于抗体的Fc区的那些生物活性,其随抗体同种型而变化。抗体效应子功能的实例包括:C1q结合和补体依赖性细胞毒性(CDC);Fc受体结合;抗体依赖性细胞介导型细胞毒性(ADCC);吞噬作用;细胞表面受体(例如B细胞受体)的下调;和B细胞活化。在本领域中已知野生型IgG4抗体的效应子功能比野生型IgG1抗体低。

[0104] 术语“Fc区”在本文中被用来定义免疫球蛋白重链的C端区域,其包含恒定区的至少一部分。该术语包括天然序列Fc区和变体Fc区。在一个实施方案中,人类IgG重链Fc区从Cys226或从Pro230延伸至重链的羧基端。然而,Fc区的C端赖氨酸(Lys447)可以存在或不存在。除非本文中另外指定,否则Fc区或恒定区中的氨基酸残基的编号是按照Kabat等人,Sequences of Proteins of Immunological Interest,第5版.Public Health Service,National Institutes of Health,Bethesda,MD,1991中所描述的EU编号系统(也称为EU指数)来进行。

[0105] 术语“全长抗体”、“完整抗体”和“全抗体”在本文中可互换地用来指具有大体上类似于天然抗体结构的结构或具有含有本文所定义的Fc区的重链的抗体。

[0106] 术语“宿主细胞”、“宿主细胞系”和“宿主细胞培养物”可互换使用,并且是指已将外源核酸引入其中的细胞,包括这类细胞的后代。宿主细胞包括“转化体”和“转化细胞”,其包括初级转化细胞及由其衍生的后代,而不考虑传代数。后代可能在核酸含量上与亲本细胞并非完全相同,而是可以含有突变。本文包括具有如在初始转化细胞中筛选或选择的相同功能或生物活性的突变体后代。

[0107] “免疫缀合物”是与一种或多种异源性分子(包括但不限于另一种治疗剂)缀合的抗体。

[0108] “分离的”核酸是指已经从其天然环境的组分中分离出的核酸分子。分离的核酸包括核酸分子,其包含在通常含有该核酸分子的细胞中,但该核酸分子存在于染色体外或其所处的染色体位置不同于其天然染色体位置。

[0109] “分离的编码抗Aβ抗体的核酸”是指编码抗体重链和轻链(或其片段)的一个或多个核酸分子,包括在单个载体或不同载体中的这类核酸分子和存在于宿主细胞中的一个或多个位置的这类核酸分子。

[0110] 如本文所使用,术语“早期阿尔茨海默氏病”或“早期AD”(例如,“诊断为早期AD的患者”或“罹患早期AD的患者”)包括具有由AD所引起的轻度认知障碍如记忆缺陷的患者和具有AD生物标记的患者例如淀粉样蛋白阳性患者。

[0111] 如本文所使用,术语“轻度阿尔茨海默氏病”或“轻度AD”(例如,“诊断为轻度AD的患者”)是指特征在于20至26的MMSE评分的AD阶段。

[0112] 如本文所使用,术语“轻到中度阿尔茨海默氏病”或“轻到中度AD”包括轻度和中度AD二者,并且其特征在于18至26的MMSE评分。

[0113] 如本文所使用,术语“中度阿尔茨海默氏病”或“中度AD”(例如,“诊断为中度AD的患者”)是指特征在于18至19的MMSE评分的AD阶段。

[0114] “裸抗体”是指未缀合至异源部分(例如另一个治疗部分)或放射性标记的抗体。该裸抗体可以存在于药物制剂中。

[0115] “天然抗体”是指具有不同结构的天然存在的免疫球蛋白分子。例如,天然IgG抗体是约150,000道尔顿的异四聚体糖蛋白,它由通过二硫键结合的两个相同轻链和两个相同重链组成。从N端至C端,每个重链具有可变区(VH),也称为可变重域或重链可变结构域,接着是三个恒定结构域(CH1、CH2和CH3)。类似地,从N端至C端,每个轻链具有可变区(VL),也称为可变轻域或轻链可变结构域,接着是恒定轻(CL)结构域。抗体的轻链基于其恒定结构域的氨基酸序列可以归于两种类型(称为卡帕(κ)和拉姆达(λ))中的一种。

[0116] 术语“包装插页”是用来指通常包含在治疗产品的商业包装中的说明书,其含有关于适应症、用法、剂量、施用、联合疗法、禁忌症的信息和/或关于使用这类治疗产品的警告。术语“包装插页”还用来指通常包含在诊断产品的商业包装中的说明书,其含有关于预期用途、测试原理、试剂的制备和操作、样本收集和制备、分析的校准和分析程序、性能和精度数据如分析的灵敏度和特异性的信息。

[0117] 关于参照多肽序列的“百分比(%)氨基酸序列同一性”的定义为在比对序列并在必要时引入缺口以获取最大百分比序列同一性后,且不将任何保守性替代视为序列同一性的部分时,候选序列中与参照多肽序列中的氨基酸残基相同的氨基酸残基的百分比。为测定百分比氨基酸序列同一性目的进行的比对可以以本领域技术范围内的多种方式来实现,例如使用可公开获得的计算机软件如BLAST、BLAST-2、ALIGN或Megalign(DNASTAR)软件。本领域技术人员可以决定用于比对序列的适宜参数,包括在被比较序列的全长里实现最大比对所需要的任何算法。然而,就本文的目的而言,使用序列比较计算机程序ALIGN-2来生成%氨基酸序列同一性值。ALIGN-2序列比较计算机程序由Genentech,Inc.创作,并且源代码已与用户文档一起提交到美国版权局(U.S.Copyright Office),Washington D.C.,20559,其被登记在美国版权登记号TXU510087下。ALIGN-2程序可从Genentech,Inc.,South San Francisco,California公开获得,或可以从源代码汇编。ALIGN-2程序应当汇编用于UNIX操作系统,包括数字UNIX V4.0D。所有序列比较参数均由ALIGN-2程序设定且不改变。

[0118] 在采用ALIGN-2进行氨基酸序列比较的情况下,给定氨基酸序列A对/与/相对于给定氨基酸序列B的%氨基酸序列同一性(或者这可以用短语表示为对/与/相对于给定氨基酸序列B具有或包含特定%氨基酸序列同一性的给定氨基酸序列A)计算如下:

[0119] 100乘以分数(X/Y)

[0120] 其中X是由序列比对程序ALIGN-2在所述程序对A和B的比对中评为相同匹配的氨基酸残基数,而其中Y是B中氨基酸残基的总数。应当了解,当氨基酸序列A的长度不等于氨基酸序列B的长度时,A对B的%氨基酸序列同一性将不等于B对A的%氨基酸序列同一性。除非另外明确说明,否则本文中使用的所有%氨基酸序列同一性值是如在前一段中所描述的

那样使用ALIGN-2计算机程序所获得。

[0121] 术语“药物制剂”和“药物组合物”在本文中可互换使用并且是指这样的制剂，其呈现使得包含在其中的活性成分的生物活性有效的形式，且不包含对将施用该制剂的受试者具有不可接受的毒性的其它组分。

[0122] “药学上可接受的载剂”是指药物制剂中除活性成分以外的成分，其对受试者无毒性。药学上可接受的载剂包括但不限于缓冲剂、赋形剂、稳定剂或防腐剂。

[0123] 如本文所使用，术语“载体”是指能够使其连接的另一种核酸增殖的核酸分子。该术语包括作为自我复制核酸结构的载体，以及掺入它所引入的宿主细胞的基因组中的载体。某些载体能够指导与其操作性连接的核酸的表达。此类载体在本文中被称为“表达载体”。

[0124] “成像剂”是具有一种或多种允许直接或间接检测到它的存在和/或位置的性质的化合物。此类成像剂的实例包括并入允许检测的标记部分的蛋白质和小分子化合物。

[0125] “标记”是与待用于检测或成像的分子偶联的标记物。此类标记的实例包括：放射性标记、荧光团、发色团或亲和标签。在一个是实施方案中，所述标记是用于医疗成像的放射性标记例如Tc99m或I123，或用于核磁共振(NMR)成像(又称为磁共振成像，mri)的自旋标记诸如碘-123、碘-131、铟-111、氟-19、碳-13、氮-15、氧-17、钆、锰、铁等。

[0126] 方法和组合物

[0127] 本公开提供了用于治疗、预后、选择和/或鉴定有淀粉样变性的风险或具有淀粉样变性的患者的组合物和方法。在一个方面，本发明是部分基于改良的治疗方法。

[0128] 在某些实施方案中，提供了结合Aβ的抗体。本发明的抗体可用于例如诊断或治疗阿尔茨海默氏病(“AD”)和其它疾病。

[0129] 示例性抗体

[0130] 在一个方面，本发明提供了结合Aβ的分离抗体。在某些实施方案中，本发明提供了能够以良好的亲和力结合人类Aβ的单体、低聚物和原纤维形式的抗Aβ抗体。在一个实施方案中，抗Aβ抗体是结合在Aβ的残基13-24内的Aβ表位的抗体。在一个这样的实施方案中，所述抗体是克雷内治单抗。

[0131] 在一个实施方案中，所述抗体包含在SEQ ID NO:5中列出的重链氨基酸序列以及在SEQ ID NO:9中列出的轻链氨基酸序列。在另一个实施方案中，所述抗体包含在SEQ ID NO:5中列出的氨基酸序列的氨基酸1-112的重链可变区以及在SEQ ID NO:9中列出的氨基酸序列的氨基酸1-112的轻链可变区。在另一个实施方案中，所述抗体包含SEQ ID NO:5和SEQ ID NO:9的HVR序列。在另一个实施方案中，所述抗体包含与SEQ ID NO:5和SEQ ID NO:9的HVR序列95％、96％、97％、98％或99％或更多相同的HVR序列。

[0132] 在任何以上实施方案中，抗Aβ抗体是人源化的。在一个实施方案中，抗Aβ抗体包含如在任何以上实施方案中的HVR，并且进一步包含受体人类框架，例如人类免疫球蛋白框架或人类共有框架。

[0133] 在另一个方面，抗Aβ抗体包含与SEQ ID NO:5的氨基酸序列的氨基酸1-112具有至少90％、91％、92％、93％、94％、95％、96％、97％、98％、99％或100％序列同一性的重链可变结构域(VH)序列。在某些实施方案中，具有至少90％、91％、92％、93％、94％、95％、96％、97％、98％或99％同一性的VH序列含有相对于参考序列的替代(例如，保守替代)、插入或缺

失,但是包含该序列的抗Aβ抗体保留结合Aβ的能力。在某些实施方案中,SEQ ID NO:5中总共有1至10个氨基酸已经被替代、插入和/或删除。在某些实施方案中,替代、插入或缺失发生在HVR以外的区域中(即,在FR中)。任选地,抗Aβ抗体包含SEQ ID NO:5中的VH序列,包括该序列的翻译后修饰。

[0134] 在另一个方面,提供了抗Aβ抗体,其中该抗体包含与SEQ ID NO:9的氨基酸序列的氨基酸1-112具有至少90%、91%、92%、93%、94%、95%、96%、97%、98%、99%或100%序列同一性的轻链可变结构域(VL)。在某些实施方案中,具有至少90%、91%、92%、93%、94%、95%、96%、97%、98%或99%同一性的VL序列含有相对于参考序列的替代(例如,保守替代)、插入或缺失,但是包含该序列的抗Aβ抗体保留结合Aβ的能力。在某些实施方案中,SEQ ID NO:9中总共有1至10个氨基酸已经被替代、插入和/或删除。在某些实施方案中,替代、插入或缺失发生在HVR以外的区域中(即,在FR中)。任选地,抗Aβ抗体包含SEQ ID NO:9中的VL序列,包括该序列的翻译后修饰。

[0135] 在另一个方面,提供了抗Aβ抗体,其中所述抗体包含如在任何上文所提供的实施方案中的VH和如在任何上文所提供的实施方案中的VL。

[0136] 在另一方面,本发明提供了与本文所提供的抗Aβ抗体结合相同的表位的抗体。例如,在某些实施方案中,提供了与包含SEQ ID NO:5中的VH序列和SEQ ID NO:9中的VL序列的抗Aβ抗体结合相同的表位的抗体。

[0137] 在本发明的另一个方面,根据任何以上实施方案的抗Aβ抗体是单克隆抗体,包括嵌合、人源化或人类抗体。在一个实施方案中,抗Aβ抗体是抗体片段,例如Fv、Fab、Fab'、scFv、双抗体或F(ab')2片段。在另一个实施方案中,抗体是全长抗体,例如,本文所定义的"完整IgG4"抗体或其它抗体类别或同种型。在某些实施方案中,抗体是双特异性抗体。

[0138] 在另一方面,根据任何以上实施方案的抗Aβ抗体可以并入如以下部分1-7中所描述的任何特征中的一个或组合。

[0139] 在一个实施方案中,抗Aβ抗体包含含有氨基酸序列SEQ ID NO:6的HVR-L1、含有氨基酸序列SEQ ID NO:7的HVR-L2、含有氨基酸序列SEQ ID NO:8的HVR-L3、含有氨基酸序列SEQ ID NO:2的HVR-H1、含有氨基酸序列SEQ ID NO:3的HVR-H2和含有氨基酸序列SEQ ID NO:4的HVR-H3。

[0140] 在另一个实施方案中,所述抗体包含SEQ ID NO:5和SEQ ID NO:9的重链和轻链序列。

[0141] 在另一个实施方案中,所述抗体包含SEQ ID NO:5和SEQ ID NO:9中的可变区序列。

[0142] 在任何以上实施方案中,抗Aβ抗体可以是人源化的。在一个实施方案中,抗Aβ抗体包含如在任何以上实施方案中的HVR,并且进一步包含受体人类框架,例如人类免疫球蛋白框架或人类共有框架。

[0143] 1.抗体亲和力

[0144] 在某些实施方案中,本文提供的抗体具有≤1μM、≤100nM、≤10nM、≤1nM、≤0.1nM、≤0.01nM或≤0.001nM(例如$10^{-8}$M或更小,例如$10^{-8}$M至$10^{-13}$M,例如,$10^{-9}$M至$10^{-13}$M)的解离常数(Kd)。

[0145] 在一个实施方案中,通过放射性标记抗原结合分析(RIA)测量Kd,该抗原结合分析

是如以下分析所描述使用受关注抗体的Fab型式和其抗原而进行。通过在未标记抗原的滴定系列的存在下用最小浓度的($^{125}$I)标记抗原平衡Fab，然后用抗Fab抗体涂层板捕捉结合抗原来测量Fab对抗原的溶液结合亲和力(参见例如，Chen等人，J.Mol.Biol.293:865-881(1999))。为了建立分析条件，用含在50mM碳酸钠(pH 9.6)中的5μg/ml捕捉用抗Fab抗体(Cappel Labs)涂覆MICROTITER®多孔板(Thermo Scientific)过夜，且随后在室温(约23℃)下用含在PBS中的2%(w/v)胎牛血清封闭2-5小时。在非吸附板(Nunc#269620)中，将100pM或26pM[125I]-抗原与受关注Fab的连续稀释液混合(例如，与Presta等人，Cancer Res.57:4593-4599(1997)中的抗VEGF抗体的评估一致)。然后过夜培养受关注的Fab；然而，培养可以持续更长时间(例如，约65小时)以确保达到平衡。之后，将混合物转移到捕捉板用于在室温下培养(例如，持续1小时)。然后移除溶液并用含在PBS中的0.1％聚山梨醇酯20(TWEEN-20®)冲洗板8次。当板已经干燥时，添加150μl/孔闪烁剂(MICROSCINT-20TM；Packard)，并在TOPCOUNT TMγ计数器(Packard)上对板计数10分钟。选择产生小于或等于最大结合的20％的每个Fab的浓度用于竞争结合分析。

[0146] 根据另一个实施方案，使用固定化抗原CM5芯片，以～10个反应单位(RU)，在25℃下使用BIACORE®-2000或BIACORE®-3000(BLAcore,Inc.,Piscataway,NJ)，利用表面等离振子共振分析来测量Kd。简而言之，根据供应商的说明书用N-乙基-N'(3-二甲氨基丙基)-碳化二亚胺盐酸盐(EDC)和N-羟基琥珀酰亚胺(NHS)激活羧甲基化葡聚糖生物传感器芯片(CM5,BIACORE,Inc.)。将抗原用10mM乙酸钠(pH 4.8)稀释到5μg/ml(～0.2μM)，然后以5μl/分钟的流速注射，以获得约10个反应单位(RU)的偶联蛋白。在注射抗原后，注射1M乙醇胺以封闭未反应的基团。对于动力学测量来说，在25℃下将Fab的两倍连续稀释液(0.78nM至500nM)以约25pl/min的流速注射到含0.05％聚山梨醇酯20(TWEEN-20TM)表面活性剂(PBST)的PBS中。使用简单的一对一Langmuir结合模型(BIACORE ®Evaluation Software版本3.2)，通过同时拟合结合和解离传感图来计算结合速率(kon)和解离速率(koff)。平衡解离常数(Kd)被计算为比率koff/kon。参见例如，Chen等人，J.Mol.Biol.293:865-881(1999)。如果通过以上的表面等离振子共振分析测得结合速率超过$10^6 M^{-1}s^{-1}$，那么可以使用荧光淬灭技术来测定结合速率，即如在分光计例如配备了断流装置的分光光度计(Aviv Instruments)或8000系列SLM-AMINCO TM分光光度计(ThermoSpectronic)中用搅拌比色杯所测量，在浓度渐增的抗原的存在下，测量PBS pH 7.2中的20nM抗原抗体(Fab形式)于25℃下的荧光发射强度(激发＝295nm；发射＝340nm，16nm带通)的升高或降低。

[0147] 2.抗体片段

[0148] 在某些实施方案中，本文提供的抗体是抗体片段。抗体片段包括但不限于Fab、Fab'、Fab'-SH、F(ab')2、Fv和scFv片段以及下文描述的其它片段。关于某些抗体片段的综述，参见Hudson等人，Nat.Med.9:129-134(2003)。关于scFv片段的综述，参见例如Pluckthün,The Pharmacology of Monoclonal Antibodies，第113卷，Rosenburg和Moore编辑，(Springer-Verlag,New York)，第269-315页(1994)；另外参见WO 93/16185；以及美国专利号5,571,894和5,587,458。关于包含补救受体结合表位残基且具有增加的体内半衰期的Fab和F(ab')2片段的讨论，参见美国专利号5,869,046。

[0149] 双抗体是具有两个抗原结合位点的抗体片段，可以是二价或双特异性。参见例如EP 404,097；WO 1993/01161；Hudson等人，Nat.Med.9:129-134(2003)；和Hollinger等人，

Proc.Natl.Acad.Sci.USA 90:6444-6448(1993)。在Hudson等人,Nat.Med.9:129-134(2003)中还描述了三抗体和四抗体。

[0150] 单结构域抗体是包含抗体的整个或部分重链可变结构域或整个或部分轻链可变结构域的抗体片段。在某些实施方案中,单结构域抗体是人类单结构域抗体(Domantis,Inc.,Waltham,MA;参见例如美国专利号6,248,516B1)。在某些实施方案中,两个或更多个单结构域抗体可以结合在一起形成具有多价亲和力的免疫球蛋白构建体(即,第一单结构域抗体的N末端或C末端可以稠合或以其它方式连接至第二单结构域抗体的N末端或C末端)。

[0151] 可以通过各种技术来生成抗体片段,这些技术包括但不限于如本文所描述的完整抗体的蛋白水解消化以及通过重组宿主细胞生成(例如,大肠杆菌(E.coli)或噬菌体)。

[0152] 3.嵌合和人源化抗体

[0153] 在某些实施方案中,本文提供的抗体是嵌合抗体。某些嵌合抗体描述于例如美国专利号4,816,567和Morrison等人,Proc.Natl.Acad.Sci.USA,81:6851-6855(1984)中。在一个实例中,嵌合抗体包括非人类可变区(例如,源自于小鼠、大鼠、仓鼠、兔子或非人类灵长类动物如猴的可变区)和人类恒定区。在另一个实例中,嵌合抗体是其中类或亚类已从亲本抗体发生改变的一个“类别切换”抗体。嵌合抗体包括其抗原结合片段。

[0154] 在某些实施方案中,嵌合抗体是人源化抗体。通常,非人类抗体被人源化以降低对人类的免疫原性,同时保留亲本非人类抗体的特异性和亲和力。通常,人源化抗体包含一个或多个可变结构域,其中HVR例如CDR(或其部分)衍生自非人类抗体且FR(或其部分)衍生自人类抗体序列。人源化抗体任选还将包含人类恒定区的至少一部分。在一些实施方案中,人源化抗体中的一些FR残基被来自非人类抗体(例如,衍生HVR残基的抗体)的相应残基取代,例如用于恢复或提高抗体特异性或亲和力。

[0155] 人源化抗体和其形成方法综述于例如Almagro和Fransson,Front.Biosci.13:1619-1633(2008)中,并且进一步描述于例如Riechmann等人,Nature 332:323-329(1988);Queen等人,Proc.Acad.Sci.USA 86:10029-10033(1989);美国专利号5,821,337、7,527,791、6,982,321和7,087,409;Kashmiri等人,Methods 36:25-34(2005)(描述SDR(a-CDR)嫁接);Padlan,Mol.Immunol.28:489-498(1991)(描述“表面重塑”);Dall'Acqua等人,Methods 36:43-60(2005)(描述“FR重排”);以及Osbourn等人,Methods 36:61-68(2005)和Klimka等人,Br.J.Cancer,83:252-260(2000)(描述用于FR重排的“导向选择”方法)中。

[0156] 可以用于人源化的人类框架区包括但不限于:使用“最佳拟合”(best-fit)方法选择的框架区(参见例如Sims等人J.Immunol.151:2296(1993));衍生自人类抗体的特定亚组的轻链或重链可变区的共有序列的框架区(参见例如Carter等人,Proc.Natl.Acad.Sci.USA,89:4285(1992);和Presta等人,J.Immunol.,151:2623(1993));人成熟(体细胞突变型)框架区或人生殖系框架区(参见例如,Almagro和Fransson,Front.Biosci.13:1619-1633(2008));和衍生自筛选FR文库的框架区(参见例如,Baca等人,J.Biol.Chem.272:10678-10684(1997)和Rosok等人,J.Biol.Chem.271:22611-22618(1996))。

[0157] 4.人类抗体

[0158] 在某些实施方案中,本文提供的抗体是人类抗体。可以使用本领域中已知的各种

技术来生成人类抗体。人类抗体一般地描述在van Dijk和van de Winkel，Curr.Opin.Pharmacol.5:368-74(2001)以及Lonberg,Curr.Opin.Immunol.20:450-459(2008)中。

[0159] 可以通过向转基因动物施用免疫原来制备人类抗体，该转基因动物已经被修饰成响应抗原攻击而生成完整人类抗体或具有人可变区的完整抗体。此类动物通常含有所有或部分人免疫球蛋白基因座，其置换内源性免疫球蛋白基因座，或者其在染色体外存在或随机整合入动物的染色体中。在此类转基因小鼠中，一般已经将内源性免疫球蛋白基因座灭活。关于从转基因动物获得人类抗体的方法的综述，参见Lonberg,Nat.Biotech.23:1117-1125(2005)。还参见例如美国专利号6,075,181和6,150,584，其描述XENOMOUSE™技术；美国专利号5,770,429，其描述HUMAB®技术；美国专利号7,041,870，其描述K-MMOUSE®技术；以及美国专利申请公开号US 2007/0061900，其描述VELOCIMOUSE®技术)。可以例如通过与不同人恒定区组合来进一步修饰来自由此类动物生成的完整抗体的人可变区。

[0160] 还可以通过基于杂交瘤的方法来生成人类抗体。已经描述了用于生成人单克隆抗体的人骨髓瘤和小鼠-人杂交骨髓瘤细胞系。(参见例如，Kozbor J.Immunol.,133:3001(1984)；Brodeur等人，Monoclonal Antibody Production Techniques and Applications，第51-63页(Marcel Dekker,Inc.,New York,1987)；和Boerner等人，J.Immunol.,147:86(1991)。)经由人B细胞杂交瘤技术生成的人类抗体还描述于Li等人，Proc.Natl.Acad.Sci.USA,103:3557-3562(2006)中。其它方法包括描述于例如美国专利号7,189,826(其描述从杂交瘤细胞系生成单克隆人类IgM抗体)和Ni,Xiandai Mianyixue,26(4):265-268(2006)(其描述人-人杂交瘤)中的那些方法。人类杂交瘤技术(Trioma技术)还描述于Vollmers和Brandlein,Histology and Histopathology,20(3):927-937(2005)以及Vollmers和Brandlein,Methods and Findings in Experimental and Clinical Pharmacology,27(3):185-91(2005)中。

[0161] 还可以通过分离选自人衍生型噬菌体展示文库的Fv克隆可变结构域序列来生成人类抗体。然后，可以将此类可变结构域序列与所需的人恒定结构域组合。下文描述了自抗体文库选择人类抗体的技术。

[0162] 5.文库衍生的抗体

[0163] 可以通过在组合文库中筛选具有所需的一种或多种活性的抗体来分离本发明的抗体。例如，用于生成噬菌体展示文库并对此类文库筛选拥有所需结合特征的抗体的多种方法是本领域中已知的。此类方法综述于例如Hoogenboom等人，Methods in Molecular Biology 178:1-37(O'Brien等人，编辑，Human Press,Totowa,NJ,2001)中并且进一步描述于例如McCafferty等人，Nature 348:552-554；Clackson等人，Nature 352:624-628(1991)；Marks等人，J.Mol.Biol.222:581-597(1992)；Marks和Bradbury,Methods in Molecular Biology 248:161-175(Lo编辑，Human Press,Totowa,NJ,2003)；Sidhu等人，J.Mol.Biol.338(2):299-310(2004)；Lee等人，J.Mol.Biol.340(5):1073-1093(2004)；Fellouse,Proc.Natl.Acad.Sci.USA 101(34):12467-12472(2004)；和Lee等人，J.Immunol.Methods 284(1-2):119-132(2004)中。

[0164] 在某些噬菌体展示方法中，将VH和VL基因的全集分别通过聚合酶链式反应(PCR)

克隆，并在噬菌体文库中随机重组，然后可以对噬菌体文库筛选抗原结合噬菌体，如Winter等人，Ann.Rev.Immunol.，12:433-455(1994)中所描述。噬菌体通常以单链Fv(scFv)片段或以Fab片段展示抗体片段。来自免疫来源的文库提供针对免疫原的高亲和力抗体，而不需要构建杂交瘤。或者，可以(例如自人类)克隆天然全集以在没有任何免疫的情况下提供针对一系列非自身和还有自身抗原的单一来源抗体，如由Griffiths等人，EMBO J,12:725-734(1993)所描述。最后，还可以通过自干细胞克隆未重排V基因区段并使用含有随机序列的PCR引物编码高度可变的CDR3区并在体外实现重排来合成生成天然文库，如由Hoogenboom和Winter,J.Mol.Biol.,227:381-388(1992)所描述。描述人类抗体噬菌体文库的专利公开包括例如：美国专利号5,750,373和美国专利公开号2005/0079574、2005/0119455、2005/0266000、2007/0117126、2007/0160598、2007/0237764、2007/0292936和2009/0002360。

[0165] 从人类抗体文库分离的抗体或抗体片段被视为本文中的人类抗体或人类抗体片段。

[0166] 6.多特异性抗体

[0167] 在某些实施方案中，本文所提供的抗体是多特异性抗体，例如双特异性抗体。多特异性抗体是对至少两个不同位点具有结合特异性的单克隆抗体。在某些实施方案中，结合特异性之一是针对Aβ，而另一种是针对任何其它抗原。在某些实施方案中，双特异性抗体可以结合Aβ的两个不同表位。双特异性抗体还可以用于将细胞毒性剂定位于细胞。双特异性抗体可以制备成全长抗体或抗体片段。

[0168] 用于生成多特异性抗体的技术包括但不限于具有不同特异性的两个免疫球蛋白重链-轻链对的重组共表达(参见Milstein和Cuello,Nature 305:537(1983))、WO 93/08829和Traunecker等人，EMBO J.10:3655(1991))和“结进孔”(knob-in-hole)工程化(参见例如美国专利号5,731,168)。还可以通过下列方法来制备多特异性抗体：用于生成抗体Fc-异二聚体分子的工程化静电操纵效应(WO 2009/089004A1)；交联两个或更多个抗体或片段(参见例如，美国专利号4,676,980和Brennan等人，Science,229:81(1985))；使用亮氨酸拉链生成双特异性抗体(参见例如Kostelny等人，J.Immunol.，148(5):1547-1553(1992))；使用用于生成双特异性抗体片段的"双抗体"技术(参见例如Hollinger等人，Proc.Natl.Acad.Sci.USA,90:6444-6448(1993))；和使用单链Fv(sFv)二聚体(参见例如Gruber等人，J.Immunol.，152:5368(1994))；以及如例如Tutt等人，J.Immunol.147:60(1991)中所述制备三特异性抗体。

[0169] 本文中还包括具有三个或更多个功能性抗原结合位点的工程化抗体，包括“章鱼抗体”(参见例如US 2006/0025576A1)。

[0170] 本文中的抗体或片段还包括“双重作用FAb”或“DAF”，其包含结合Aβ以及另一种不同抗原的抗原结合位点(参见例如US2008/0069820)。

[0171] 7.抗体变体

[0172] 在某些实施方案中，预期了本文所提供的抗体的氨基酸序列变体。例如，可以期望改善抗体的结合亲和力和/或其它生物特性。可以通过将适当的修饰引入编码抗体的核苷酸序列中或通过肽合成来制备抗体的氨基酸序列变体。此类修饰包括例如对抗体的氨基酸序列内的残基进行删除和/或插入和/或替代。可以进行删除、插入和替代的任何组合以得到最终的构建体，只要最终的构建体拥有所需的特征例如抗原结合。

[0173] 替代、插入和缺失变体

[0174] 在某些实施方案中，提供了具有一个或多个氨基酸替代的抗体变体。替代诱变的受关注位点包括HVR和FR。保守替代在表1中的“保守替代”的标题下示出。更实质的变化提供在表1中的“示例性替代”标题下，并且如下文参照氨基酸侧链类别所进一步描述。可以将氨基酸替代引入受关注抗体中，并且对产物筛选所需的活性，例如保留/改善的抗原结合、降低的免疫原性或改善的ADCC或CDC。

[0175] 表1

[0176]

| 原始残基 | 示例性替代 | 保守性替代 |
|---|---|---|
| Ala (A) | Val; Leu; Ile | Val |
| Arg (R) | Lys; Gln; Asn | Lys |
| Asn (N) | Gln; His; Asp, Lys; Arg | Gln |
| Asp (D) | Glu; Asn | Glu |
| Cys (C) | Ser; Ala | Ser |
| Gln (Q) | Asn; Glu | Asn |
| Glu (E) | Asp; Gln | Asp |
| Gly (G) | Ala | Ala |
| His (H) | Asn; Gln; Lys; Arg | Arg |
| Ile (I) | Leu; Val; Met; Ala; Phe; 正亮氨酸 | Leu |
| Leu (L) | 正亮氨酸; Ile; Val; Met; Ala; Phe | Ile |
| Lys (K) | Arg; Gln; Asn | Arg |
| Met (M) | Leu; Phe; Ile | Leu |
| Phe (F) | Trp; Leu; Val; Ile; Ala; Tyr | Tyr |
| Pro (P) | Ala | Ala |
| Ser (S) | Thr | Thr |
| Thr (T) | Val; Ser | Ser |
| Trp (W) | Tyr; Phe | Tyr |
| Tyr (Y) | Trp; Phe; Thr; Ser | Phe |
| Val (V) | Ile; Leu; Met; Phe; Ala; 正亮氨酸 | Leu |

[0177] 根据共同的侧链特性，氨基酸可以分组如下：

[0178] (1)疏水性：正亮氨酸、Met、Ala、Val、Leu、Ile；

[0179] (2)中性亲水性：Cys、Ser、Thr、Asn、Gln；

[0180] (3)酸性：Asp、Glu；

[0181] (4)碱性：His、Lys、Arg；

[0182] (5)影响链取向的残基：Gly、Pro；

[0183] (6)芳香族:Trp、Tyr、Phe。

[0184] 非保守替代将需要用这些类别之一的成员替换另一个类别。

[0185] 一种类型的替代变体涉及替代亲本抗体(例如人源化或人类抗体)的一个或多个高变区残基。通常,被选择用于进一步研究的所得变体相对于亲本抗体将具有改变的(例如改善的)某些生物性质(例如增加的亲和力、降低的免疫原性)和/或将具有亲本抗体的大体上保留的某些生物性质。一种示例性替代变体是亲和力成熟抗体。在某些实施方案中,亲和力成熟抗体对靶抗原将具有纳摩尔浓度或甚至皮摩尔浓度的亲和力。亲和力成熟抗体是通过本领域中已知的程序而产生,包括例如使用基于噬菌体展示的亲和力成熟技术(诸如本文所描述的那些)。简而言之,使一个或多个HVR残基突变并在噬菌体上展示变体抗体,且针对特定生物活性(例如结合亲和力)进行筛选。还已知其它程序。Marks等人,Bio/Technology 10:779-783(1992)描述了通过VH和VL结构域重排进行亲和力成熟。Barbas等人,Proc Nat.Acad.Sci,USA 91:3809-3813(1994);Schier等人,Gene 169:147-155(1996);Yelton等人,J.Immunol.155:1994-2004(1995);Jackson等人,J.Immunol.154(7):3310-9(1995);和Hawkins等人,J.Mol.Biol.226:889-896(1992)描述了HVR和/或框架残基的随机诱变。

[0186] 可以在HVR中进行改变(例如替代)以例如改善抗体亲和力。可以在HVR“热点”(即由在体细胞成熟过程中经历高频突变的密码子编码的残基)(参见例如Chowdhury,Methods Mol.Biol.207:179-196(2008))和/或SDR(a-CDR)中进行此类改变,并测试所得变体VH或VL的结合亲和力。通过从二级文库中构建和再选择实现的亲和力成熟已描述于例如Hoogenboom等人,Methods in Molecular Biology 178:1-37(O'Brien等人编辑,Human Press,Totowa,NJ,(2001))中。在亲和力成熟的一些实施方案中,通过多种方法(例如易错PCR、链改组或寡核苷酸定向诱变)中的任一种将多样性引入被选择用于突变的可变基因中。然后创建二级文库。然后筛选该文库以鉴定具有所需亲和力的任何抗体变体。引入多样性的另一种方法涉及HVR引导方法,其中使几个HVR残基随机化(例如一次4-6个残基)。涉及抗原结合的HVR残基可以例如使用丙氨酸扫描诱变或建模明确地识别。特别地,经常靶向CDR-H3和CDR-L3。

[0187] 在某些实施方案中,替代、插入或缺失可以在一个或多个HVR中发生,只要此类改变不大体上降低抗体结合抗原的能力。例如,可以在HVR中进行大体上不减小结合亲和力的保守性改变(例如本文提供的保守性替代)。此类改变可以在HVR“热点”或SDR之外。在上文提供的变体VH和VL的某些实施方案中,每个HVR未改变或含有不多于一个、两个或三个氨基酸替代。

[0188] 用于鉴定抗体中可以被选为诱变目标的残基或区域的一种有用方法被称为“丙氨酸扫描诱变”,如由Cunningham和Wells(1989)Science,244:1081-1085所描述。在该方法中,鉴定残基或靶残基组(例如带电残基如arg、asp、his、Iys和glu)并用中性或带负电荷的氨基酸(例如丙氨酸或聚丙氨酸)替换,以测定是否影响抗体与抗原的相互作用。可以在对最初替代显示功能敏感性的氨基酸位置引入另外的替代。或者或另外,使用抗原-抗体复合物的晶体结构来鉴定抗体与抗原之间的接触点。可以靶向或消除此类接触残基和邻近残基作为替代的候选。可以筛选变体以确定它们是否含有所需性质。

[0189] 氨基酸序列插入包括氨基和/或羧基末端融合,其长度范围是从一个残基至包含

一百个或更多个残基的多肽，以及单个或多个氨基酸残基的序列内插入。末端插入的实例包括具有N端甲硫氨酰残基的抗体。抗体分子的其它插入变体包括使抗体的N或C端与酶(例如对于ADEPT)或增加抗体的血清半衰期的多肽融合。

[0190] 糖基化变体

[0191] 在某些实施方案中，改变本文所提供的抗体以提高或降低抗体糖基化的程度。可以通过改变氨基酸序列以创建或消除一个或多个糖基化位点来方便地实现对抗体的糖基化位点的添加或删除。

[0192] 在抗体包含Fc区的情况下，可以改变其附接的碳水化合物。由哺乳动物细胞生成的天然抗体通常包含分支的双触角寡糖，其一般通过N-连接附接至Fc区的CH2结构域的Asn297。参见例如，Wright等人TIBTECH 15:26-32(1997)。寡糖可以包括各种碳水化合物，例如，甘露糖、N-乙酰葡糖胺(GlcNAc)、半乳糖和唾液酸，以及附接至双触角寡糖结构的“主干”中的GlcNAc的岩藻糖。在一些实施方案中，可以对本发明抗体中的寡糖进行修饰以创建具有某些改善的特性的抗体变体。

[0193] 在一个实施方案中，提供了抗体变体，其具有缺少附接(直接或间接)至Fc区的岩藻糖的碳水化合物结构。例如，此类抗体中的岩藻糖量可以是1％至80％、1％至65％、5％至65％或20％至40％。通过相对于附接至Asn297的所有糖结构(例如，复合、杂合和高甘露糖结构)的总和，计算Asn297处的糖链内岩藻糖的平均量来测定岩藻糖量，如通过MALDI-TOF质谱法所测量(如例如WO2008/077546中所描述)。Asn297是指位于Fc区中的约第297位的天冬酰胺残基(Fc区残基的Eu编号方式)；然而，Asn297还可以由于抗体中的微小序列变异而位于第297位的约±3个氨基酸的上游或下游处，即在第294位和第300位之间。此类岩藻糖基化变体可以具有改善的ADCC功能。参见例如美国专利公开号US 2003/0157108(Presta, L.)；US 2004/0093621(Kyowa Hakko Kogyo Co.,Ltd)。涉及“脱岩藻糖基化”或“岩藻糖缺乏”抗体变体的出版物的实例包括：US 2003/0157108；WO 2000/61739；WO 2001/29246；US 2003/0115614；US 2002/0164328；US 2004/0093621；US 2004/0132140；US 2004/0110704；US 2004/0110282；US 2004/0109865；WO 2003/085119；WO 2003/084570；WO 2005/035586；WO 2005/035778；WO 2005/053742；WO 2002/031140；Okazaki等人J.Mol.Biol.336:1239-1249(2004)；Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004)。能够生成脱岩藻糖基化抗体的细胞系的实例包括缺乏蛋白质岩藻糖基化的Lec 13CHO细胞(Ripka等人Arch.Biochem.Biophys.249:533-545(1986)；美国专利申请号US 2003/0157108A1, Presta,L；和WO 2004/056312 A1,Adams等人，尤其是实施例11)，以及敲除细胞系如α-1,6-岩藻糖基转移酶基因FUT8敲除的CHO细胞(参见例如，Yamane-Ohnuki等人Biotech.Bioeng.87:614(2004)；Kanda,Y.等人,Biotechnol.Bioeng.,94(4):680-688(2006)；和WO2003/085107)。

[0194] 进一步提供了具有两分型寡糖的抗体变体，例如其中附接至抗体Fc区的双触角寡糖通过GlcNAc二等分。此类抗体变体可以具有降低的岩藻糖基化和/或改善的ADCC功能。此类抗体变体的实例描述于例如WO 2003/011878(Jean-Mairet等人)；美国专利号6,602,684(Umana等人)和US 2005/0123546(Umana等人)中。还提供了在附接至Fc区的寡糖中具有至少一个半乳糖残基的抗体变体。此类抗体变体可以具有改善的CDC功能。此类抗体变体描述于例如WO 1997/30087(Patel等人)；WO 1998/58964(Raju,S.)；和WO 1999/22764(Raju,

S.)中。

[0195] Fc区变体

[0196] 在某些实施方案中,可以将一处或多处氨基酸修饰引入本文所提供的抗体的Fc区中,由此生成Fc区变体。Fc区变体可以包含在一个或多个氨基酸位置包含氨基酸修饰(例如替代)的人Fc区序列(例如人IgG1、IgG2、IgG3或IgG4Fc区)。

[0197] 在某些实施方案中,本发明预期拥有一些但不是所有效应子功能的抗体变体,该效应子功能使其成为如下应用的期望候选物:其中抗体的体内半衰期是重要的,而某些效应子功能(诸如补体和ADCC)是不必要或有害的。可以进行体外和/或体内细胞毒性分析以确认CDC和/或ADCC活性的降低/消减。例如,可以进行Fc受体(FcR)结合分析以确保抗体缺乏Fc R结合(因此可能缺乏ADCC活性),但是保留FcRn结合能力。用于介导ADCC的原始细胞NK细胞仅表达FcλRIII,而单核细胞表达FcλRI、FcλRII和FcλRIII。造血细胞上的FcR表达汇总于Ravetch和Kinet,Annu.Rev.Immunol.9:457-492(1991)的第464页上的表3中。评估目标分子的ADCC活性的体外分析的非限制性实例描述于美国专利号5,500,362(参见例如Hellstrom,I.等人Proc.Nat'l Acad.Sci.USA 83:7059-7063(1986))和Hellstrom,I等人,Proc.Nat'l Acad.Sci.USA 82:1499-1502(1985);5,821,337(参见Bruggemann,M.等人,J.Exp.Med.166:1351-1361(1987))中。或者,可以采用非放射性分析方法(参见例如用于流式细胞术的ACTI™非放射性细胞毒性分析(CellTechnology,Inc.Mountain View,CA;和CytoTox96®非放射性细胞毒性分析(Promega,Madison,WI)。用于此类分析的效应细胞包括外周血单核细胞(PBMC)和天然杀伤(NK)细胞。或者或另外,可以在体内评估受关注分子的ADCC活性,诸如在动物模型诸如Clynes等人Proc.Nat'l Acad.Sci.USA 95:652-656(1998)中公开的动物模型中。还可以进行C1q结合分析以确认抗体不能结合C1q并且因此缺乏CDC活性。参见例如WO 2006/029879和WO 2005/100402中的C1q和C3c结合ELISA。为了评估补体激活,可以进行CDC分析(参见例如,Gazzano-Santoro等人,J.Immunol.Methods 202:163(1996);Cragg,M.S.等人,Blood 101:1045-1052(2003);以及Cragg,M.S.和M.J.Glennie,Blood 103:2738-2743(2004))。还可以使用本领域中已知的方法来进行FcRn结合和体内清除/半衰期测定(参见例如,Petkova,S.B.等人,Int'l.Immunol.18(12):1759-1769(2006))。

[0198] 效应子功能降低的抗体包括具有Fc区残基238、265、269、270、297、327和329中的一个或多个的替代的那些抗体(美国专利号6,737,056)。此类Fc突变体包括在氨基酸位置265、269、270、297和327中的两处或更多处具有替代的Fc突变体,包括残基265和297被替代为丙氨酸的所谓"DANA"Fc突变体(美国专利号7,332,581)。

[0199] 描述了对FcR的结合有所改善或减弱的某些抗体变体。(参见例如美国专利号6,737,056;WO 2004/056312和Shields等人,J.Biol.Chem.9(2):6591-6604(2001)。)

[0200] 在某些实施方案中,抗体变体包含具有改善ADCC的一处或多处氨基酸替代例如在Fc区的位置298、333和/或334(残基的EU编号方式)处的替代的Fc区。

[0201] 在一些实施方案中,对Fc区做出改变,其导致改变(即,改善或减弱)的C1q结合和/或补体依赖性细胞毒性(CDC),例如,如美国专利号6,194,551、WO 99/51642和Idusogie等人,J.Immunol.164:4178-4184(2000)中所描述。

[0202] 具有增加的半衰期和改善的对新生儿Fc受体(FcRn)的结合的抗体描述于US2005/

0014934A1(Hinton等人)中，新生儿Fc受体负责将母体IgG转移至胎儿(Guyer等人，J.Immunol.117:587(1976)和Kim等人，J.Immunol.24:249(1994))。那些抗体包含其中具有改善Fc区对FcRn的结合的一个或多个替代的Fc区。此类Fc变体包括在Fc区残基238、256、265、272、286、303、305、307、311、312、317、340、356、360、362、376、378、380、382、413、424或434中的一个或多个处具有替代，例如Fc区残基434的替代的那些变体(美国专利号7,371,826)。关于Fc区变体的其它实例，还参见Duncan和Winter,Nature 322:738-40(1988)；美国专利号5,648,260；美国专利号5,624,821；和WO 94/29351。

[0203] 半胱氨酸工程化抗体变体

[0204] 在某些实施方案中，可以期望形成半胱氨酸工程化抗体，例如“thioMAb”，其中抗体的一个或多个残基被半胱氨酸残基替代。在特定实施方案中，被替代的残基存在于抗体的可接近位点处。通过用半胱氨酸替代那些残基，反应性硫醇基团由此定位于抗体的可接近位点并且可以用于使抗体与其它部分诸如药物部分或连接子团-药物部分缀合，以创建如本文中进一步描述的免疫缀合物。在某些实施方案中，可以用半胱氨酸替代下列残基中的任何一个或多个：轻链的V205(Kabat编号方式)；重链的A118(EU编号方式)；和重链Fc区的S400(EU编号方式)。可以如例如美国专利号7,521,541所描述来生成半胱氨酸工程化抗体。

[0205] 抗体衍生物

[0206] 在某些实施方案中，可以进一步修饰本文所提供的抗体以含有本领域已知的且易于获得的额外非蛋白质部分。适于抗体衍生化的部分包括但不限于水溶性聚合物。水溶性聚合物的非限制性实例包括但不限于聚乙二醇(PEG)、乙二醇/丙二醇共聚物、羧甲基纤维素、葡聚糖、聚乙烯醇、聚乙烯吡咯烷酮、聚-1,3-二氧戊环、聚-1,3,6-三噁烷、乙烯/马来酸酐共聚物、聚氨基酸(均聚物或随机共聚物)、和葡聚糖或聚(n-乙烯吡咯烷酮)聚乙二醇、丙二醇均聚物、环氧丙烷/环氧乙烷共聚物、聚氧乙烯化多元醇(例如甘油)、聚乙烯醇和其混合物。聚乙二醇丙醛由于其在水中的稳定性而可以在生产中具有优势。聚合物可以具有任何分子量，且可以是支链或无支链型。附接至抗体上的聚合物数量可以变化，而且如果附接了超过一个聚合物，则它们可以是相同或不同的分子。一般而言，可以基于多种考虑来确定用于衍生化的聚合物的数量和/或类型，这些考虑包括但不限于抗体要改进的具体性质或功能、抗体衍生物是否将用于限定条件下的治疗等。

[0207] 在另一个实施方案中，提供了抗体与可以通过暴露于辐射而选择性加热的非蛋白质部分的缀合物。在一个实施方案中，非蛋白质部分是碳纳米管(Kam等人，Proc.Natl.Acad.Sci.USA 102:11600-11605(2005))。辐射可以具有任何波长，且包括但不限于这样的波长，其对普通细胞没有损害，但是将非蛋白质部分加热至抗体-非蛋白质部分附近的细胞被杀死的温度。

[0208] 重组方法和组合物

[0209] 可以使用重组方法和组合物产生抗体，例如如美国专利号4,816,567中所描述。在一个实施方案中，提供了编码本文所描述的抗Aβ抗体的分离核酸。所述核酸可以编码包含所述抗体的VL的氨基酸序列和/或包含所述抗体的VH的氨基酸序列(例如，抗体的轻链和/或重链)。在另一个实施方案中，提供一个或多个包含所述核酸的载体(例如，表达载体)。在另一个实施方案中，提供包含所述核酸的宿主细胞。在一个这类实施方案中，宿主细胞包含

(例如,已被其转化):(1)含有核酸的载体,该核酸编码含有抗体的VL的氨基酸序列和含有抗体的VH的氨基酸序列;或(2)含有核酸的第一载体,该核酸编码含有抗体的VL的氨基酸序列,以及含有核酸的第二载体,该核酸编码含有抗体的VH的氨基酸序列。在一个实施方案中,宿主细胞是真核的,例如,中国仓鼠卵巢(CHO)细胞或淋巴样细胞(例如,Y0、NS0、Sp20细胞)。在一个实施方案中,提供制备抗Aβ抗体的方法,其中该方法包括在适于表达所述抗体的条件下培养如以上提供的含有编码所述抗体的核酸的宿主细胞,并任选地从宿主细胞(或宿主细胞培养基)回收抗体。

[0210] 就抗Aβ抗体的重组生成而言,分离例如如上所述的编码抗体的核酸并插入到用于在宿主细胞中进一步克隆和/或表达的一个或多个载体中。所述核酸可以使用常规程序(例如,通过使用能特异性结合编码抗体的重链和轻链的基因的寡核苷酸探针)来容易地分离和测序。

[0211] 适用于抗体编码载体的克隆或表达的宿主细胞包括本文所描述的原核或真核细胞。例如,抗体可以在细菌中产生,特别是当不需要糖基化和Fc效应子功能时。关于抗体片段和多肽在细菌中的表达,参见例如美国专利号5,648,237、5,789,199和5,840,523。(还参见Charlton,Methods in Molecular Biology,第248卷(B.K.C.Lo编辑.,Humana Press,Totowa,NJ,2003),第245-254页,其描述了抗体片段在大肠杆菌中的表达)。表达后,抗体可以从可溶性部分中的细菌细胞浆中分离并且可以被进一步纯化。

[0212] 除了原核生物以外,真核微生物例如丝状真菌或酵母是适用于抗体编码载体的克隆或表达宿主,包括糖基化路径已经被"人源化"的真菌和酵母菌株,从而产生具有部分或完全人类糖基化模式的抗体。参见Gerngross,Nat.Biotech.22:1409-1414(2004),和Li等人,Nat.Biotech.24:210-215(2006)。

[0213] 适用于表达糖基化抗体的宿主细胞还源于多细胞生物体(无脊椎动物和脊椎动物)。无脊椎动物细胞的实例包括植物和昆虫细胞。已经鉴别出可以与昆虫细胞共同使用,特别是用于转染草地贪夜蛾(Spodoptera frugiperda)细胞的大量杆状病毒菌株。

[0214] 植物细胞培养物也可以用作宿主。参见例如美国专利号5,959,177、6,040,498、6,420,548、7,125,978和6,417,429(描述了用于在转基因植物中产生抗体的PLANTIBODIES™技术)。

[0215] 脊椎动物细胞也可以用作宿主。例如,适于悬浮生长的哺乳动物细胞系可以是有用的。有用的哺乳动物宿主细胞系的其它实例是通过SV40(COS-7)转化的猴肾CV1细胞系;人类胚胎肾细胞系(如例如Graham等人,J.Gen Virol.36:59(1977)中所描述的293或293细胞);乳仓鼠肾细胞(BHK);小鼠睾丸支持细胞(如例如Mather,Biol.Reprod.23:243-251(1980)中所描述的TM4细胞);猴肾细胞(CV1);非洲绿猴肾细胞(VERO-76);人宫颈癌细胞(HELA);犬肾细胞(MDCK;布法罗大鼠(buffalo rat)肝细胞(BRL 3A);人肺细胞(W138);人肝细胞(Hep G2);小鼠乳腺肿瘤(MMT 060562);如例如Mather等人,Annals N.Y.Acad.Sci.383:44-68(1982)中所描述的TRI细胞;MRC 5细胞;和FS4细胞。其它有用的哺乳动物宿主细胞系包括中国仓鼠卵巢(CHO)细胞,包括DHFR-CHO细胞(Urlaub等人,Proc.Natl.Acad.Sci.USA 77:4216(1980));和骨髓瘤细胞系如Y0、NS0和Sp2/0。关于适用于抗体生成的某些哺乳动物宿主细胞系的综述,参见例如Yazaki和Wu,Methods in Molecular Biology,第248卷(B.K.C.Lo编辑,Humana Press,Totowa,NJ),第255-268页

(2003)。

[0216] 分析

[0217] 本文所提供的抗Aβ抗体可以通过本领域中已知的各种分析针对其物理/化学性质和/或生物活性来识别、筛选或表征。

[0218] 结合分析和其它分析

[0219] 在一个方面,测试本发明抗体的抗原结合活性,例如通过已知的方法如ELISA、西方印迹法等。

[0220] 在另一个方面,可以利用竞争分析来识别与本发明的抗Aβ抗体竞争地结合Aβ的抗体。在某些实施方案中,这种竞争抗体结合被克雷内治单抗或本文所指定的另一种抗Aβ抗体结合的相同表位(例如线性或构象表位)。用于绘制与抗体结合的表位的详细示例性方法提供于Morris(1996)"Epitope Mapping Protocols",Methods in Molecular Biology第66卷(Humana Press,Totowa,NJ)中。

[0221] 在示例性竞争分析中,在溶液中培养呈所需形式(例如,单体、低聚物或原纤维)的固定化Aβ,所述溶液包含与Aβ结合的第一标记抗体(例如,克雷内治单抗)和待测试其与第一抗体竞争结合Aβ的能力的第二未标记抗体。第二抗体可以存在于杂交瘤上清液中。作为对照,在包含第一标记抗体但不含第二未标记抗体的溶液中培养固定化Aβ。在允许第一抗体与Aβ结合的条件下培养后,去除过量的未结合抗体,并测定与固定化Aβ相关的标记的量。如果与固定化Aβ相关的标记的量在测试样品中相对于在对照样品中大体上减少,则这指示第二抗体与第一抗体竞争结合Aβ。参见Harlow和Lane(1988)Antibodies:A Laboratory Manual第14章(Cold Spring Harbor Laboratory,Cold Spring Harbor,NY)。

[0222] 活性分析

[0223] 在一个方面,提供了用于鉴别具有生物活性(例如克雷内治单抗的生物活性)的抗Aβ抗体的分析。生物活性可以包括但不限于例如防止单体Aβ聚集成低聚Aβ,或低聚Aβ解聚成单体Aβ。还提供了在体内和/或体外具有这种生物活性的抗体。

[0224] 在某些实施方案中,测试本发明抗体的这种生物活性。

[0225] 用于诊断和检测的方法和组合物

[0226] 在某些实施方案中,本文所提供的任何抗Aβ抗体均可用于检测生物样品中Aβ的存在。如本文所使用,术语"检测"涵盖定量或定性检测。在某些实施方案中,生物样品包括细胞或组织,诸如血清、血浆、鼻拭物、痰、脑脊液、眼房水等,或从生物体获得的组织或细胞样品,诸如含有神经或脑组织的样品。

[0227] 在一个实施方案中,提供了用于诊断或检测方法中的抗Aβ抗体。在另一方面,提供了检测生物样品中Aβ的存在的方法。在某些实施方案中,方法包括在允许抗Aβ抗体与Aβ结合的条件下使生物样品与如本文所述的抗Aβ抗体接触,并检测抗Aβ抗体与Aβ之间是否形成复合物。这种方法可以是体外或体内方法。

[0228] 可以使用本发明抗体诊断的示例性病症是由淀粉样蛋白或淀粉样蛋白样蛋白引起或与其相关的疾病和病症。这些包括但不限于由单体、原纤维或聚合物状态的淀粉样蛋白样蛋白或所述三种的任意组合(包括淀粉样蛋白斑)的存在或活性引起的疾病和病症。示例性疾病包括但不限于继发性淀粉样变性和年龄相关性淀粉样变性,诸如包括但不限于下述的疾病:神经系统病症如阿尔茨海默氏病("AD")、以先天性记忆能力丧失为特征的疾病

或病状如(例如)轻度认知障碍(MCI)、路易体痴呆症、唐氏综合症、遗传性脑出血伴淀粉样变性(Dutch型)、关岛帕金森病-痴呆复合征和其它基于淀粉样蛋白样蛋白或与其相关的疾病,诸如进行性核上麻痹、多发性硬化、克雅病、帕金森病、HIV相关性痴呆、ALS(肌萎缩性侧索硬化症)、包含体肌炎(IBM)、成年发作型糖尿病、内分泌肿瘤和老年心脏淀粉样变性,以及多种眼部疾病,包括黄斑变性、玻璃疣相关性视神经病变、青光眼和由β-淀粉样蛋白沉积引起的白内障。

[0229] 在某些实施方案中,提供了标记的抗Aβ抗体。标记包括但不限于:直接检测的标记或部分(例如荧光标记、显色标记、电子致密标记、化学发光标记和放射性标记);以及例如通过酶促反应或分子相互作用间接检测的部分,例如酶或配体。示例性标记包括但不限于:放射性同位素$^{32}P$、$^{14}C$、$^{125}I$、$^{3}H$和$^{131}I$;荧光团例如稀土螯合物或荧光素和其衍生物、罗丹明(rhodamine)和其衍生物、丹磺酰、伞酮(umbelliferone)、虫荧光素酶例如萤火虫荧光素酶和细菌荧光素酶(美国专利号4,737,456)、虫荧光素、2,3-二氢酞嗪二酮、辣根过氧化物酶(HRP)、碱性磷酸酶、β-半乳糖苷酶、葡糖淀粉酶、溶菌酶;糖氧化酶例如葡萄糖氧化酶、半乳糖氧化酶和葡萄糖-6-磷酸脱氢酶;杂环氧化酶例如尿酸酶和黄嘌呤氧化酶,其与采用过氧化氢来氧化染料前体的酶例如HRP、乳过氧化物酶或微过氧化物酶偶联;生物素/亲和素、自旋标记、噬菌体标记、稳定自由基等。

[0230] 药物制剂

[0231] 本文所描述的抗Aβ抗体的药物制剂是通过将具有所需纯度的此类抗体或分子与一种或多种可选的药学上可接受的载剂(Remington's Pharmaceutical Sciences第16版,Osol,A.编辑(1980))混合成冻干制剂或水溶液形式而制备。药学上可接受的载剂通常在所用的剂量和浓度下对接受者无毒,且包括但不限于:缓冲液,例如磷酸盐、柠檬酸盐和其它有机酸;抗氧化剂,包括抗坏血酸和蛋氨酸;防腐剂(例如十八烷基二甲基苄基氯化铵、氯化六烃季铵、苯扎氯铵、苄索氯铵、苯酚、丁醇或苄醇;对羟基苯甲酸烷基酯,例如对羟基苯甲酸甲酯或丙酯;邻苯二酚;间苯二酚;环己醇;3-戊醇和间甲酚);低分子量(少于约10个残基)多肽;蛋白质,例如血清白蛋白、明胶或免疫球蛋白;亲水性聚合物例如聚乙烯吡咯烷酮;氨基酸例如甘氨酸、谷氨酰胺、天冬酰胺、组氨酸、精氨酸或赖氨酸;单糖、二糖和其它碳水化合物,包括葡萄糖、甘露糖或糊精;螯合剂例如EDTA;糖类例如蔗糖、甘露糖、海藻糖或山梨醇;成盐抗衡离子例如钠;金属复合物(例如Zn-蛋白质复合物);和/或非离子表面活性剂例如聚乙二醇(PEG)。本文中的示例性药学上可接受的载剂进一步包括间质性药物分散剂例如可溶性中性-活性透明质酸酶糖蛋白(sHASEGP),例如人可溶性PH-20透明质酸酶糖蛋白,例如rHuPH20(HYLENEX®,Baxter International,Inc.)。某些示例性sHASEGP和包括rHuPH20的使用方法描述于美国专利公开号2005/0260186和2006/0104968中。在一个方面,将sHASEGP与一种或多种额外的糖胺聚糖例如软骨素酶组合。

[0232] 在一个实施方案中,可以在精氨酸缓冲液中配制本发明的抗体。在一个方面,精氨酸缓冲液可以是精氨酸琥珀酸盐缓冲液。在一个这样的方面,精氨酸琥珀酸盐缓冲液的浓度可以是50mM或更高。在另一个这样的方面,精氨酸琥珀酸盐缓冲液的浓度可以是100mM或更高。在另一个这样的方面,精氨酸琥珀酸盐缓冲液的浓度可以是150mM或更高。在另一个这样的方面,精氨酸琥珀酸盐缓冲液的浓度可以是200mM或更高。在另一个方面,精氨酸缓冲液制剂可以进一步包含表面活性剂。在另一个这样的方面,表面活性剂是聚山梨醇酯。在

另一个这样的方面，聚山梨醇酯是聚山梨醇酯20。在另一个这样的方面，聚山梨醇酯20在制剂中的浓度为0.1％或更小。在另一个这样的方面，聚山梨醇酯20在制剂中的浓度为0.05％或更小。在另一个方面，精氨酸缓冲液制剂的pH在4.5与7.0之间。在另一个方面，精氨酸缓冲液制剂的pH在5.0与6.5之间。在另一个方面，精氨酸缓冲液制剂的pH在5.0与6.0之间。在另一个方面，精氨酸缓冲液制剂的pH为5.5。在任何前述实施方案和方面中，本发明的抗体可以是克雷内治单抗。

[0233] 示例性冻干抗体制剂描述于美国专利号6,267,958中。水性抗体制剂包括描述于美国专利号6,171,586和WO2006/044908中的那些，后者的制剂包括组氨酸-乙酸盐缓冲液。

[0234] 本文中的制剂还可以包含多于一种治疗特定适应症所需要的活性成分，优选那些具有不互相产生不利影响的互补活性的活性成分。例如，可能需要进一步提供一种或多种化合物来预防或治疗阿尔茨海默氏病的症状。此类活性成分适合以对于预期目的有效的量组合存在。

[0235] 可以将活性成分包埋在例如通过凝聚技术或通过界面聚合制备的微胶囊(例如分别为羟甲基纤维素或明胶微胶囊和聚(甲基丙烯酸甲酯)微胶囊)中、胶体药物递送系统(例如，脂质体、白蛋白微球、微乳液、纳米颗粒和纳米胶囊)中或粗乳液中。此类技术公开于Remington's Pharmaceutical Sciences第16版,Osol,A.编辑(1980)中。

[0236] 可以制备持续释放制剂。持续释放制剂的合适实例包括包含抗体的固体疏水聚合物的半渗透基质，所述基质呈成形制品形式，例如薄膜或微胶囊。

[0237] 用于体内施用的制剂一般是无菌的。可以例如通过经无菌滤膜过滤容易地实现无菌性。

[0238] 治疗方法和组合物

[0239] 如本文中所示，克雷内治单抗的静脉内施用减少罹患AD的患者中的疾病进展。具体来说，患有轻到中度AD的患者(包括患有轻度AD的患者和ApoE4阳性患者)以及具有常见于被诊断为AD的患者中的脑淀粉样蛋白负荷的患者在经克雷内治单抗治疗时相较于经安慰剂治疗时展现出降低的认知衰退速率。所述疾病越温和(基于渐增的MMSE评分)，治疗组中的衰退减少相比于安慰剂组越大。这些结果通过由克雷内治单抗接触的目标的其它迹象(包括在脑脊液中检测到的Aβ水平的增加和淀粉样蛋白在脑中累积的减少)进一步证实。此外，抗体的相对高的剂量-15mg/kg-没有增加已在其它抗Aβ抗体的试验中观察到的ARIA型不良事件的发生率。

[0240] 因此，在一个实施方案中，本发明的抗体被用于治疗AD，包括轻到中度AD、轻度AD和早期AD。在另一个实施方案中，本发明的抗体被用于治疗淀粉样变性。在一个这样的实施方案中，淀粉样变性是轻度认知障碍。在另一个这样的实施方案中，淀粉样变性是唐氏综合症。在另一个这样的实施方案中，淀粉样变性是遗传性脑出血伴淀粉样变性(Dutch型)。在另一个这样的实施方案中，淀粉样变性是关岛帕金森病-痴呆复合征。在另一个这样的实施方案中，淀粉样变性是涉及眼睛中的玻璃疣或其它淀粉样沉积物的眼部疾病。在一个方面，眼部疾病是黄斑变性。在另一个方面，眼部疾病是玻璃疣相关性视神经病变。在另一个方面，眼部疾病是青光眼。在另一个方面，眼部疾病是白内障。在任何前述实施方案和方面中，本发明的抗体可以是克雷内治单抗。

[0241] 通常首先评估患者中的一种或多种淀粉样变性的存在，然后确定本发明的抗体用

于治疗该患者的合适性。作为一个非限制性实例，可以使用“NINCDS-ADRDA”(神经和交际障碍和中风-阿尔茨海默氏病相关病症评估)标准诊断患者中的AD。参见McKhann等人，1984，Neurology 34:939-44。待施用本发明的一种或多种抗体的潜在患者还可以被测试一种或多种遗传标记的存在或不存在，所述遗传标记可能使该患者倾向于具有(i)该患者经历一种或多种淀粉样变性的较高或较低的可能性，或(ii)该患者在本发明抗体的施用过程中经历一个或多个不良事件或副作用的较高或较低的可能性。作为一个非限制性实例，已知携带ApoE4等位基因的患者比缺乏该等位基因的患者具有大体上更高的发展AD的风险(Saunders等人，Neurology 1993;43:1467-72;Prekumar等人，Am.J.Pathol.1996;148:2083-95)，并且此类患者在贝平珠单抗(另一个抗Aβ抗体)的临床试验中观察到的ARIA型不良事件中被不成比例地表现(Sperling等人，Alzheimer's&Dementia 2011,7:367-385;Salloway等人，N.Engl.J.Med.2014,370:322-333)。

[0242] 在一些实施方案中，本发明的抗体被用于治疗患者中的轻到中度AD。患者可以是ApoE4阳性或ApoE4阴性。在一些实施方案中，本发明的抗体被用于治疗轻度AD。在一些实施方案中，本发明的抗体被用于治疗罹患轻到中度AD或轻度AD的ApoE4阳性患者。在一些实施方案中，本发明的抗体被用于治疗罹患轻度AD的患者。

[0243] 在一些实施方案中，本发明的抗体被用于治疗具有20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分的患者。在一些实施方案中，患者具有22和26之间的MMSE评分。如本文中所使用，两个数字之间的MMSE评分包括在该范围的每一端的数字。例如，22和26之间的MMSE评分包括22和26的MMSE评分。

[0244] 在一些实施方案中，本发明的抗体被用于治疗“淀粉样蛋白阳性”患者，例如具有脑淀粉样蛋白沉积物(它们是被诊断为AD的患者的特征)的患者或具有阳性florbetapir PET扫描的患者。在一些实施方案中，本发明的抗体被用于减少脑淀粉样蛋白沉积物或神经炎性斑的累积(即，减少脑淀粉样蛋白负担或负荷的增加)。

[0245] 此外，本发明的抗体可用于治疗轻到中度AD而不增加ARIA-E或ARIA-H的发生率。在一些实施方案中，患者罹患轻度AD。在一些实施方案中，患者为ApoE4阳性。在一些实施方案中，患者为ApoE4阳性且罹患轻度AD。

[0246] 如本文的实施例中所证实，治疗效果在患有轻度形式的AD的患者中增强。因此，在一些实施方案中，本发明的抗体被用于治疗患有早期AD的患者。在某些实施方案中，待治疗的患者具有以下特性中的一个或多个：(a)由AD引起的轻度认知障碍(MCI)；(b)指示没有临床可检测缺陷的阿尔茨海默氏病的一种或多种生物标记；(c)使用自由和暗示选择性提醒测试(FCSRT)量化为27或更大的评分的客观记忆丧失；24-30的MMSE；(d)0.5的总体临床痴呆评级(CDR)；和(e)阳性淀粉样蛋白PET扫描(如由合格的读数器所测定)。

[0247] 本发明的抗体是以与良好的医学实践一致的方式进行配制、给药和施用。在此情况下的考虑因素包括所治疗的特定病症、所治疗的特定哺乳动物、个体受试者的临床状况、病症的起因、药剂的递送部位、施用方法、施药时间表和医疗从业人员已知的其它因素。

[0248] 施用途径

[0249] 本发明的抗体(和任何其它治疗剂)可以通过任何合适的方式来施用，包括肠胃外、肺内和鼻内施用以及如果期望用于局部治疗，病灶内施用。肠胃外输注包括肌肉内、静

脉内、动脉内、腹膜内或皮下施用。给药可以通过任何合适的途径，例如通过注射如静脉内或皮下注射，这部分取决于施用是短暂还是长期的。在一个实施方案中，皮下注射所述抗体。在另一个实施方案中，静脉注射所述抗体。在另一个实施方案中，使用注射器(例如，预填充的或未预填充)或自动注射器施用所述抗体。在另一个实施方案中，吸入所述抗体。

[0250] 给药

[0251] 对于淀粉样变性的治疗而言，本发明抗体(当单独或与一种或多种其它额外的治疗剂组合使用时)的适当剂量将取决于待治疗的疾病的具体类型、抗体的类型、疾病的严重度和病程、先前疗法、患者的临床病史和对所述抗体的反应以及主治医师的判断。在一次或一系列治疗中适宜地对患者施用所述抗体。本文预期了各种给药时间表，包括但不限于在不同时间点的单次或多次施用、推注施用和脉冲输注。

[0252] 根据疾病的类型和严重度，约0.3mg/kg至100mg/kg(例如15mg/kg-100mg/kg，或该范围内的任何剂量)的抗体可以是无论例如通过一次或多次分开施用或通过连续输注而施用给患者的初始候选剂量。取决于上述因素，一个典型的日剂量可以在从约15mg/kg至100mg/kg或更多的范围内。所述剂量可以以单剂量或分剂量(例如，30mg/kg的总剂量被分成15mg/kg的两个剂量)来施用。对于持续数周或更长的重复施用，根据状况，治疗一般会持续至出现对疾病症状的所需抑制。抗体的一个示例性剂量将在从约10mg/kg至约50mg/kg的范围内。因此，可以向患者施用约0.5mg/kg、1mg/kg、1.5mg/kg、2.0mg/kg、3mg/kg、4.0mg/kg、5mg/kg、10mg/kg、15mg/kg、20mg/kg、25mg/kg、30mg/kg、35mg/kg、40mg/kg、50mg/kg、60mg/kg、70mg/kg、80mg/kg、90mg/kg或100mg/kg的一个或多个剂量(或其任何组合)。在一些实施方案中，施用的总剂量在50mg至2500mg的范围内。可以向患者施用约50mg、约100mg、200mg、300mg、400mg、约500mg、约600mg、约700mg、约720mg、约1000mg、约1050mg、约1100mg、约1200mg、约1300mg、约1400mg、约1500mg、约1600mg、约1700mg、约1800mg、约1900mg、约2000mg、约2050mg、约2100mg、约2200mg、约2300mg、约2400mg或约2500mg的示例性剂量(或其任何组合)。此类剂量可以间歇地施用，例如每周、每两周、每三周、每四周、每个月、每两个月、每三个月或每六个月。在一些实施方案中，患者接受1至35个剂量(例如，约18个剂量的抗体)。然而，可以使用其它给药方案。这种疗法的进展可以通过常规技术和分析来监测。

[0253] 在某些实施方案中，本发明的抗体是以15mg/kg、30mg/kg、40mg/kg、45mg/kg、50mg/kg、60mg/kg的剂量或平剂量(例如300mg、500mg、700mg、800mg或更高)来施用。在一些实施方案中，通过静脉注射每2周或每4周施用所述剂量并持续一段时间。在一些实施方案中，通过皮下注射每2周或每4周施用所述剂量并持续一段时间。在某些实施方案中，所述时间段是6个月、一年、十八个月、两年、五年、十年、15年、20年或患者的一生。

[0254] 监测/评估对治疗性治疗的反应

[0255] 如本公开的方法中所用，抗体或其抗原结合片段为患者提供治疗效果或益处。在某些实施方案中，治疗益处是延迟或抑制AD的进展或临床、功能或认知衰退的减少。在一些实施方案中，治疗效果或益处反映在“患者反应”或“反应”(及其语法变型)中。患者反应可使用指示对患者的益处的任一终点来评估，包括(但不限于)(1)在一定程度上抑制疾病进展，包括减缓和完全阻止；(2)斑块数量减少或大脑淀粉样蛋白累积减少；(3)一个或多个评估度量的改良，包括(但不限于)ADAS-Cog、iADL和COR-SOB量表；(4)患者每日活动的改良；(5)脑脊髓液中一种或多种生物标记(例如Aβ)的浓度的增加；和(6)一种或多种生物指示AD

存在的标记降低。患者反应的评估还可以包括评估可能出现的可与治疗相关联的任何不良事件。

[0256] 在一个实施方案中，在利用本发明抗体的疗法过程之前、在此期间和/或之后评估患者的认知能力和日常活动。已开发许多认知和活动评价工具用于对心智功能、认知和神经功能缺陷进行评价、诊断和评分。这些工具包括(但不限于)ADAS-Cog，包括12项ADAS-Cog(ADAS-Cog12)、13项ADAS-Cog(ADAS-Cog13)、14项ADAS-Cog(ADAS-Cog14)；CDR-SOB，包括CDR判断与问题解决和CDR记忆分量；工具性日常生活活动(iADL)；和MMSE。

[0257] “ADAS-Cog”是指阿尔茨海默氏病评估量表认知子量表，即多部分认知评估。参见Rosen等人，1984，Amer.J.Psych.141:1356-1364；Mohs等人，1997，Alzheimer's Disease Assoc.Disorders 11(2):S13-S21。ADAS-Cog的数值评分越高，所测试患者的缺陷或障碍相对于另一具有低评分的个体越大。ADAS-Cog可以用作评价AD的治疗在治疗上是否有效的一种量度。ADAS-Cog评分增加指示患者的状况变差，而ADAS-Cog评分下降表示患者的状况得到改良。如本文所用，“ADAS-Cog性能衰退”或“ADAS-Cog评分增加”指示患者的状况变差且可以反映AD的进展。ADAS-Cog是评估多个认知领域的检查者实施的测试组(examiner-administered battery)，包括记忆、理解力、实践、适应和自然语言(Rosen等人，1984，Am J Psychiatr 141:1356-64；Mohs等人，1997，Alzheimer Dis Assoc Disord 11(S2):S13-S21)。ADAS-Cog是AD治疗试验中的标准主要终点(Mani 2004，Stat Med 23:305-14)。ADAS-Cog12是ADAS-Cog加上用于评价所学单词列表的回忆的10点延迟单词回忆项目的70点版本。其它ADAS-Cog量表包括ADAS-Cog13和ADAS-Cog14。

[0258] 在一些实施方案中，本文所提供的治疗方法提供认知衰退的减少，如由相对于安慰剂低至少约30％、至少约35％、至少约40或至少约45％的ADAS-Cog评分所测量。

[0259] “MMSE”是指简易智力状态检查，提供介于1与30之间的评分。参见Folstein等人，1975，J.Psychiatr.Res.12:189-98。26和更低的评分通常被认为指示缺陷。MMSE的数值评分越低，所测试患者的缺陷或障碍相对于另一具有低评分的个体越大。MMSE评分增加可以指示患者状况改良，而MMSE评分降低可表示患者状况变差。

[0260] “CDR-SOB”是指临床痴呆评定量表/得分总和。参见Hughes等人，1982.CDR-assesses 6components:memory，orientation，judgment/problem solving，community affairs，home and hobbies，and personal care。测试是对患者和照料者二者实施，且每一分量(或每一“盒(box)”)以0至3的标度进行评分。完整CDR-SOB评分基于所有6个盒的评分总和。还可以个别地针对每个盒或分量获得子评分，例如CDR/记忆或CDR/判断与问题解决。如本文所用，“CDR-SOB性能的衰退”或“CDR-SOB评分的增加”可以指示患者的状况变差且可以反映AD进展。在一些实施方案中，本文所提供的治疗方法提供CDR-SOB性能的衰退相对于安慰剂至少约30％、至少约35％或至少约40％的减少。

[0261] “iADL”是指工具性日常生活活动量表(Instrumental Activities of Daily Living scale)。参见Lawton，M.P.，和Brody，E.M.，1969，Gerontologist 9:179-186。此量表测量执行典型的日常活性的能力，例如家务、洗衣、打电话、购物、准备饭菜等。评分越低，个体在进行日常生活活动中受到的消弱越多。在一些实施方案中，本文所提供的治疗方法提供iADL量表中相对于安慰剂的至少约10％、至少约15％或至少约20％的衰退减少。

[0262] 大脑淀粉样蛋白负荷或负担可使用神经学成像技术和工具(例如使用PET(正电子

发射断层摄影)扫描)来测定。患者在(例如)施用治疗之前和之后(或在整个治疗方案过程期间以一个或多个间隔)随时间获得系列PET扫描,此可允许检测大脑中增加、降低或无变化的淀粉样蛋白负担。此技术可以进一步用于测定淀粉样蛋白累积是增加还是减少。在一些实施方案中,大脑中淀粉样蛋白沉积的检测是使用florbetapir $^{18}$F实施。在一些实施方案中,如果基于扫描的中心化视觉读出,其建立中度至频繁神经炎斑块,那么认为florbetapir PET扫描是阳性的。

[0263] 共同施用

[0264] 抗体不需要,但任选地与目前用于预防或治疗所讨论的病症或其一种或多种症状的一种或多种药剂一起配制。这类其它药剂的有效量取决于抗体在制剂中的存在量、病症或治疗的类型和上述其它因素。这些药剂通常以相同的剂量和如本文所描述的施用途径,或本文所描述的剂量的约1至99%,或者以任何剂量和通过在经验/临床上被确定为适当的任何途径来使用。本领域普通技术人员应当理解,本发明的抗体可以与任何前述化合物同时共同施用,或可以在施用任何前述化合物之前或之后施用。

[0265] 当用本发明的抗体治疗淀粉样变性时,可以共同施用神经药物。该神经药物可以选自包括但不限于以下药物的组:特异性靶向选自以下的标靶的抗体或其它结合分子(包括但不限于小分子、肽、适体或其它蛋白结合剂):β分泌酶、tau、早老素、淀粉样前体蛋白或其部分、淀粉样β肽或其低聚物或原纤维、死亡受体6(DR6)、晚期糖基化终产物的受体(RAGE)、帕金蛋白和亨廷顿蛋白;胆碱酯酶抑制剂(即,加兰他敏、多奈哌齐、卡巴拉汀和他克林);NMDA受体拮抗剂(即,美金刚胺)、单胺耗竭剂(即,丁苯喹嗪);甲磺酸二氢麦角碱;抗胆碱能抗帕金森病剂(即,普环啶、苯海拉明、三己芬迪、苯托品、比哌立登和苯海索);多巴胺能抗帕金森病剂(即,恩他卡朋、司来吉兰、普拉克索、溴隐亭、罗替戈汀、司来吉兰、罗匹尼罗、雷沙吉兰、阿朴吗啡、卡比多巴、左旋多巴、培高利特、托卡朋和金刚烷胺);丁苯喹嗪;抗炎药(包括但不限于非类固醇类抗炎药)(即吲哚美辛和上文列举的其它化合物);激素(即,雌激素、孕酮和亮丙瑞林);维生素(即,叶酸和烟酰胺);二甲弗林;高牛磺酸(即,3-氨基丙磺酸;3APS);血清素受体活性调节剂(即,扎利罗登);干扰素和糖皮质激素或皮质类固醇。在一些实施方案中,共同施用一种或多种除克雷内治单抗以外的抗Aβ抗体。这些抗Aβ抗体的非限制性实例包括苏兰珠单抗、贝平珠单抗、阿达鲁单抗和格特鲁单抗。术语“皮质类固醇”包括但不限于氟替卡松(包括丙酸氟替卡松(FP))、倍氯米松(beclometasone)、布地奈德(budesonide)、环索奈德(ciclesonide)、莫米松(mometasone)、氟尼缩松(flunisolide)、倍他米松(betamethasone)和去炎松(betamethasone)。“可吸入皮质类固醇”意指适用于通过吸入来递送的皮质类固醇。示例性可吸入皮质类固醇为氟替卡松、二丙酸倍氯米松、布地奈德、糠酸莫米松、环索奈德、氟尼缩松和曲安奈德(triamcinolone acetonide)。

[0266] 在利用本发明抗体治疗为眼部疾病或病症的淀粉样变性时,可以选择以下神经疾病药物:抗血管生成眼科用药剂(即,贝伐珠单抗(bevacizumab)、兰尼单抗(ranibizumab)和哌加他尼(pegaptanib))、眼科用青光眼药剂(即,碳酸胆碱(carbachol)、肾上腺素、地美溴铵(demecarium bromide)、阿拉可乐定(apraclonidine)、溴莫尼定(brimonidine)、布林佐胺(brinzolamide)、左布诺洛尔(levobunolol)、噻吗洛尔(timolol)、倍他洛尔(betaxolol)、多佐胺(dorzolamide)、比马前列素(bimatoprost)、卡替洛尔(carteolol)、

美替洛尔(metipranolol)、地匹福林(dipivefrin)、曲伏前列素(travoprost)和拉坦前列素(latanoprost))、碳酸酐酶抑制剂(即,醋甲唑胺(methazolamide)和乙酰唑胺(acetazolamide))、眼科用抗组胺剂(即,萘甲唑啉(naphazoline)、苯肾上腺素(phenylephrine)和四氢唑啉(tetrahydrozoline)、眼用润滑剂、眼科用类固醇(即,氟米龙(fluorometholone)、脱氢皮质醇(prednisolone)、氯替泼诺(loteprednol)、地塞米松(dexamethasone)、二氟泼尼酯(difluprednate)、利美索隆(rimexolone)、氟轻松(fluocinolone)、甲羟松(medrysone)和曲安奈德(triamcinolone)、眼科用麻醉剂(即,利多卡因(lidocaine))、丙美卡因(proparacaine)和(tetracaine))、眼科用抗感染药(即,左氧氟沙星(levofloxacin)、加替沙星(gatifloxacin)、环丙沙星(ciprofloxacin)、莫西沙星(moxifloxacin)、氯霉素(chloramphenicol)、枯草菌素/多黏杆菌素b(bacitracin/polymyxin b)、乙酰磺胺(sulfacetamide)、妥布霉素(tobramycin)、阿奇霉素(azithromycin)、贝西沙星(besifloxacin)、诺氟沙星(norfloxacin)、磺胺异噁唑(sulfisoxazole)、庆大霉素(gentamicin)、碘苷(idoxuridine)、红霉素(erythromycin)、链霉菌素(natamycin)、短杆菌素(gramicidin)、新霉素(neomycin)、氧氟沙星(ofloxacin)、曲氟尿苷(trifluridine)、更昔洛韦(ganciclovir)、阿糖腺苷(vidarabine))、眼科用抗发炎剂(即,奈帕芬胺(nepafenac)、酮洛酸(ketorolac)、氯比洛芬(flurbiprofen)、舒洛芬(suprofen)、环孢素(cyclosporine)、曲安奈德(triamcinolone)、双氯芬酸(diclofenac)和氯酚酸(bromfenac)和眼科用抗组氨剂或去充血剂(即,酮替芬(ketotifen)、奥洛他定(olopatadine)、依匹斯汀(epinastine)、萘甲唑啉(naphazoline)、色甘酸(cromolyn)、四氢唑啉(tetrahydrozoline)、吡嘧司特(pemirolast)、贝托斯汀(bepotastine)、萘甲唑啉、苯肾上腺素、奈多罗米(nedocromil)、洛度沙胺(lodoxamide)、苯肾上腺素、依美斯汀(emedastine)和氮卓斯汀(azelastine))。应该理解,以上制剂或治疗方法中的任一个都可使用本发明的免疫缀合物替代抗Aβ抗体或除抗Aβ抗体以外使用本发明的免疫缀合物实施。

[0267] 制品

[0268] 在本发明的另一个方面,提供了包含可用于治疗、预防和/或诊断上述病症的材料的制品。所述制品包括容器和位于容器上或与容器相连的标签或包装插页。合适的容器包括例如瓶子、小瓶、注射器、静脉输液袋等。容器可以由多种材料例如玻璃或塑料形成。容器内含有单独的组合物或与另一种有效治疗、预防和/或诊断病状的组合物组合的组合物,并且可以具有无菌接入口(例如容器可以为具有塞子的静脉输液袋或小瓶,塞子可被皮下注射针刺穿)。组合物中的至少一种活性剂为本发明抗体。标签或包装插页指示组合物用于治疗特别的病状。另外,所述制品可以包含(a)其中含有组合物的第一容器,其中该组合物包含本发明的抗体;和(b)其中含有组合物的第二容器,其中该组合物包含另一种细胞毒性剂或治疗剂。在本发明的这个实施方案中的制品可以进一步包括指示组合物可以用于治疗特定病状的包装说明书。或者或另外,所述制品可以进一步包含第二(或第三)容器,其包含药学上可接受的缓冲液,例如注射用抑菌水(BWFI)、磷酸盐缓冲盐水、林格氏溶液(Ringer's solution)和葡萄糖溶液。可以进一步包括就商业和用户立场而言可期望的其它材料,包括其它缓冲液、稀释剂、过滤器、针和注射器。

[0269] 应该理解,任何上述制品都可以包括本发明的免疫缀合物来代替或补充抗Aβ抗

体。

[0270] 示例性实施方案

[0271] 本文提供了用于说明的示例性实施方案。

[0272] 1.一种减少被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的功能或认知能力衰退的方法,其包括向罹患早期或轻到中度AD的患者施用能有效减缓所述患者中的功能或认知能力衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体,所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

[0273] 2.根据实施方案1所述的方法,其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0274] 3.根据权利要求1所述的方法,其中所述抗体是IgG4抗体。

[0275] 4.根据实施方案2或3所述的方法,其中所述抗体包含六个高变区(HVR),其中:

[0276] (i)HVR-H1是SEQ ID NO:2;

[0277] (ii)HVR-H2是SEQ ID NO:3;

[0278] (iii)HVR-H3是SEQ ID NO:4;

[0279] (iv)HVR-L1是SEQ ID NO:6;

[0280] (v)HVR-L2是SEQ ID NO:7;且

[0281] (vi)HVR-L3是SEQ ID NO:8。

[0282] 5.根据实施方案4所述的方法,其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0283] 6.根据实施方案5所述的方法,其中所述抗体是克雷内治单抗。

[0284] 7.根据前述实施方案中任一项所述的方法,其中通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分来评估认知能力的衰退,任选地,其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30%、至少35%、至少40%或至少45%。

[0285] 8.根据实施方案7所述的方法,其中所述患者为ApoE4阳性。

[0286] 9.根据实施方案7所述的方法,其中所述患者罹患轻度AD。

[0287] 10.根据实施方案7所述的方法,其中所述患者罹患早期AD。

[0288] 11.根据实施方案1至8中任一项所述的方法,其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

[0289] 12.根据实施方案11所述的方法,其中所述患者具有22和26之间的MMSE。

[0290] 13.根据前述实施方案中任一项所述的方法,其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

[0291] 14.根据实施方案13所述的方法,其中以至少15mg/kg的剂量施用所述抗体。

[0292] 15.根据实施方案14所述的方法,其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

[0293] 16.根据实施方案13或14所述的方法,其中经由静脉内注射施用所述抗体。

[0294] 17.根据实施方案13至16中任一项所述的方法,其中每2周、每4周、每个月、每两个

月或每六个月施用所述抗体。

[0295] 18.一种治疗早期或轻到中度AD而不增加不良事件的风险的方法,其包括向被诊断为早期或轻到中度AD的患者施用能有效治疗所述AD而不增加治疗出现的不良事件的风险的用量的人源化单克隆抗Aβ抗体,所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内,其中所述不良事件选自:(i)淀粉样蛋白相关成像异常-水肿(ARIA-E)和(ii)淀粉样蛋白相关成像异常-出血(ARIA-H)。

[0296] 19.根据实施方案18所述的方法,其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0297] 20.根据实施方案18所述的方法,其中所述抗体是IgG4抗体。

[0298] 21.根据实施方案19所述的方法,其中所述抗体包含六个高变区(HVR),其中:

[0299] (i)HVR-H1是SEQ ID NO:2;

[0300] (ii)HVR-H2是SEQ ID NO:3;

[0301] (iii)HVR-H3是SEQ ID NO:4;

[0302] (iv)HVR-L1是SEQ ID NO:6;

[0303] (v)HVR-L2是SEQ ID NO:7;且

[0304] (vi)HVR-L3是SEQ ID NO:8。

[0305] 22.根据实施方案21所述的方法,其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0306] 23.根据实施方案22所述的方法,其中所述抗体是克雷内治单抗。

[0307] 24.根据实施方案18至23中任一项所述的方法,其中所述患者为ApoE4阳性。

[0308] 25.根据实施方案18至23中任一项所述的方法,其中所述不良事件为ARIA-E。

[0309] 26.根据实施方案25所述的方法,其中如果检测到治疗出现的ARIA-E,则停止施用所述抗体并且任选地施用针对ARIA-E的治疗。

[0310] 27.根据实施方案26所述的方法,其进一步包括在解决所述ARIA-E后恢复施用所述抗体,其中所述抗体以低于停止施用前的剂量施用。

[0311] 28.根据实施方案18所述的方法,其中如果在用所述抗体进行治疗期间在所述患者中检测到一个或多个新的ARIA-E,则不再施用抗体,并且任选地向所述患者施用皮质类固醇。

[0312] 29.根据实施方案28所述的方法,其中所述患者为ApoE4阳性。

[0313] 30.一种减少被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的功能或认知能力衰退的方法,其包括向罹患早期或轻到中度AD的ApoE4阳性患者施用能有效减缓所述患者中的功能或认知能力衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体,所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

[0314] 31.根据实施方案30所述的方法,其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0315] 32.根据实施方案30所述的方法,其中所述抗体是IgG4抗体。

[0316] 33.根据实施方案31或32所述的方法,其中所述抗体包含六个高变区(HVR),其中:

[0317] (i)HVR-H1是SEQ ID NO:2;

[0318] (ii)HVR-H2是SEQ ID NO:3;

[0319] (iii)HVR-H3是SEQ ID NO:4；

[0320] (iv)HVR-L1是SEQ ID NO:6；

[0321] (v)HVR-L2是SEQ ID NO:7；且

[0322] (vi)HVR-L3是SEQ ID NO:8。

[0323] 34.根据实施方案33所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0324] 35.根据实施方案34所述的方法，其中所述抗体是克雷内治单抗。

[0325] 36.根据实施方案30至35中任一项所述的方法，其中通过使用ADAS-Cog12、ADAS-Cog13或ADAS-Cog14测试确定在施用所述抗体之前和之后的患者评分来评估认知能力的衰退，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

[0326] 37.根据实施方案36所述的方法，其中所述患者具有轻度AD。

[0327] 38.根据实施方案36所述的方法，其中所述患者具有早期AD。

[0328] 39.根据实施方案30至37中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

[0329] 40.根据实施方案39所述的方法，其中所述患者具有22和26之间的MMSE评分。

[0330] 41.根据实施方案30至39中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

[0331] 42.根据实施方案41所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

[0332] 43根据实施方案42所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

[0333] 44.根据实施方案41或42所述的方法，其中经由静脉内注射施用所述抗体。

[0334] 45.根据实施方案41至44中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

[0335] 46.一种治疗早期或轻到中度AD而不增加不良事件的风险的方法，其包括向被诊断为早期或轻到中度AD的ApoE4阳性患者施用能有效治疗所述AD而不增加治疗出现的不良事件的风险的用量的人源化单克隆抗Aβ抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内，其中所述不良事件选自：(i)淀粉样蛋白相关成像异常-水肿(ARIA-E)和(ii)淀粉样蛋白相关成像异常-出血(ARIA-H)。

[0336] 47.根据实施方案46所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0337] 48.根据实施方案46所述的方法，其中所述抗体是IgG4抗体。

[0338] 49.根据实施方案47所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

[0339] (i)HVR-H1是SEQ ID NO:2；

[0340] (ii)HVR-H2是SEQ ID NO:3；

[0341] (iii)HVR-H3是SEQ ID NO:4；

[0342] (iv)HVR-L1是SEQ ID NO:6；

[0343] (v)HVR-L2是SEQ ID NO:7；且

[0344] (vi)HVR-L3是SEQ ID NO:8。

[0345] 50.根据实施方案49所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0346] 51.根据实施方案50所述的方法，其中所述抗体是克雷内治单抗。

[0347] 52根据实施方案46至51中任一项所述的方法，其中所述不良事件为ARIA-E。

[0348] 53.根据实施方案52所述的方法，其中如果检测到治疗出现的ARIA-E，则停止施用所述抗体并且任选地施用针对ARIA-E的治疗。

[0349] 54.根据实施方案53所述的方法，其进一步包括在解决所述ARIA-E后恢复施用所述抗体，任选地包括以低于停止施用前的剂量恢复施用所述抗体。

[0350] 55.根据实施方案46所述的方法，其中如果在用所述抗体进行治疗期间在所述患者中检测到一个或多个新的ARIA-E，则不再施用抗体，并且任选地向所述患者施用皮质类固醇。

[0351] 56.根据前述实施方案中任一项所述的方法，其中同时用选自由以下所组成的组的一种或多种药剂治疗所述患者：特异性结合标靶的治疗剂；胆碱酯酶抑制剂；NMDA受体拮抗剂；单胺耗竭剂；甲磺酸二氢麦角碱；抗胆碱能抗帕金森病剂；多巴胺能抗帕金森病剂；丁苯喹嗪；抗炎剂；激素；维生素；二甲弗林；高牛磺酸；血清素受体活性调节剂；干扰素和糖皮质激素；除克雷内治单抗以外的抗Aβ抗体；抗生素；抗病毒剂。

[0352] 57.根据实施方案56所述的方法，其中所述药剂是胆碱酯酶抑制剂。

[0353] 58.根据实施方案57所述的方法，其中所述胆碱酯酶抑制剂选自由加兰他敏、多奈哌齐、卡巴拉汀和他克林组成的组。

[0354] 59.根据实施方案56所述的方法，其中所述药剂是NMDA受体拮抗剂。

[0355] 60.根据实施方案59所述的方法，其中所述NMDA受体拮抗剂是美金刚胺或其盐。

[0356] 61.根据实施方案56所述的方法，其中所述药剂是特异性结合标靶的治疗剂且所述标靶选自由以下所组成的组：β分泌酶、tau、早老素、淀粉样前体蛋白或其部分、淀粉样β肽或其低聚物或原纤维、死亡受体6(DR6)、晚期糖基化终产物的受体(RAGE)、帕金蛋白和亨廷顿蛋白。

[0357] 62.根据实施方案56所述的方法，其中所述药剂是单胺耗竭剂，任选为丁苯喹嗪。

[0358] 63.根据实施方案56所述的方法，其中所述药剂是选自由以下所组成的组的抗胆碱能抗帕金森病剂：普环啶、苯海拉明、三己芬迪、苯托品、比哌立登和苯海索。

[0359] 64.根据实施方案56所述的方法，其中所述药剂是选自由以下所组成的组的多巴胺能抗帕金森病剂：恩他卡朋、司来吉兰、普拉克索、溴隐亭、罗替戈汀、司来吉兰、罗匹尼罗、雷沙吉兰、阿朴吗啡、卡比多巴、左旋多巴、培高利特、托卡朋和金刚烷胺。

[0360] 65.根据实施方案56所述的方法，其中所述药剂是选自由非类固醇类抗炎药和吲哚美辛组成的组的抗炎剂。

[0361] 66.根据实施方案56所述的方法，其中所述药剂是选自由雌激素、孕酮和亮丙瑞林组成的组的激素。

[0362] 67.根据实施方案56所述的方法，其中所述药剂是选自由叶酸和烟酰胺组成的组的维生素。

[0363] 68.根据实施方案56所述的方法，其中所述药剂是高牛磺酸，其为3-氨基丙磺酸或

3APS。

[0364] 69.根据实施方案56所述的方法，其中所述药剂是扎利罗登。

[0365] 70.一种减缓被诊断为早期或轻到中度阿尔茨海默氏病(AD)的患者中的临床衰退的方法，其包括向罹患早期或轻到中度AD的患者施用能有效减缓所述患者中的衰退的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

[0366] 71.根据实施方案70所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0367] 72.根据实施方案70所述的方法，其中所述抗体是IgG4抗体。

[0368] 73.根据实施方案71或72所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

[0369] (i)HVR-H1是SEQ ID NO:2；

[0370] (ii)HVR-H2是SEQ ID NO:3；

[0371] (iii)HVR-H3是SEQ ID NO:4；

[0372] (iv)HVR-L1是SEQ ID NO:6；

[0373] (v)HVR-L2是SEQ ID NO:7；且

[0374] (vi)HVR-L3是SEQ ID NO:8。

[0375] 74.根据实施方案73所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0376] 75.根据实施方案74所述的方法，其中所述抗体是克雷内治单抗。

[0377] 76.根据实施方案70至75中任一项所述的方法，其进一步包括通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分所评估的认知能力衰退，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

[0378] 77.根据实施方案76所述的方法，其中所述患者为ApoE4阳性。

[0379] 78.根据实施方案76所述的方法，其中所述患者罹患轻度AD。

[0380] 79.根据实施方案76所述的方法，其中所述患者罹患早期AD。

[0381] 80.根据实施方案70至78中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

[0382] 81.根据实施方案80所述的方法，其中所述患者具有22和26之间的MMSE评分。

[0383] 82.根据实施方案70至80中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

[0384] 83.根据实施方案82所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

[0385] 84.根据实施方案83所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

[0386] 85.根据实施方案82或83所述的方法，其中经由静脉内注射施用所述抗体。

[0387] 86.根据实施方案82至85中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

[0388] 87.一种治疗受试者中的早期或轻度AD的方法，其包括向罹患早期或轻度AD的患者施用能有效治疗所述AD的用量的人源化单克隆抗淀粉样蛋白β(Aβ)抗体，所述抗体结合在淀粉样蛋白β(1-42)(SEQ ID NO:1)的残基13和24内。

[0389] 88.根据实施方案87所述的方法，其中所述抗体能够结合淀粉样蛋白β的低聚和单体形式。

[0390] 89.根据实施方案87所述的方法，其中所述抗体是IgG4抗体。

[0391] 90.根据实施方案88或89所述的方法，其中所述抗体包含六个高变区(HVR)，其中：

[0392] (i)HVR-H1是SEQ ID NO:2；

[0393] (ii)HVR-H2是SEQ ID NO:3；

[0394] (iii)HVR-H3是SEQ ID NO:4；

[0395] (iv)HVR-L1是SEQ ID NO:6；

[0396] (v)HVR-L2是SEQ ID NO:7；且

[0397] (vi)HVR-L3是SEQ ID NO:8。

[0398] 91.根据实施方案90所述的方法，其中所述抗体包含具有SEQ ID NO:5的氨基酸序列的重链和具有SEQ ID NO:9的氨基酸序列的轻链。

[0399] 92.根据实施方案91所述的方法，其中所述抗体是克雷内治单抗。

[0400] 93.根据实施方案87至92中任一项所述的方法，其中所述用量能有效减少认知能力的衰退，所述认知能力的衰退是通过使用12项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)、13项阿尔茨海默氏病评估量表-认知(ADAS-Cog13)或14项阿尔茨海默氏病评估量表-认知(ADAS-Cog12)测试确定在施用所述抗体之前和之后的患者评分来评估，任选地，其中如通过ADAS-Cog所测量的认知衰退的减少相对于安慰剂为至少30％、至少35％、至少40％或至少45％。

[0401] 94.根据实施方案93所述的方法，其中所述患者为ApoE4阳性。

[0402] 95.根据实施方案87至94中任一项所述的方法，其中所述患者在治疗开始前具有至少20、20和30之间、20和26之间、24和30之间、21和26之间、22和26之间、22和28之间、23和26之间、24和26之间或25和26之间的MMSE评分。

[0403] 96.根据实施方案95所述的方法，其中所述患者具有22和26之间的MMSE评分。

[0404] 97.根据实施方案87至95中任一项所述的方法，其中以10mg/kg至100mg/kg患者体重的剂量施用所述抗体。

[0405] 98.根据实施方案97所述的方法，其中以至少15mg/kg的剂量施用所述抗体。

[0406] 99.根据实施方案98所述的方法，其中以15mg/kg、30mg/kg、45mg/kg、50mg/kg或60mg/kg的剂量施用所述抗体。

[0407] 100.根据实施方案97或98所述的方法，其中经由静脉内注射施用所述抗体。

[0408] 101.根据实施方案97至100中任一项所述的方法，其中每2周、每4周、每个月、每两个月或每六个月施用所述抗体。

[0409] 102.根据实施方案70至101中任一项所述的方法，其中同时用选自由以下所组成的组的一种或多种药剂治疗所述患者：特异性结合标靶的治疗剂；胆碱酯酶抑制剂；NMDA受体拮抗剂；单胺耗竭剂；甲磺酸二氢麦角碱；抗胆碱能抗帕金森病剂；多巴胺能抗帕金森病剂；丁苯喹嗪；抗炎剂；激素；维生素；二甲弗林；高牛磺酸；血清素受体活性调节剂；干扰素

和糖皮质激素;抗Aβ抗体;抗生素;抗病毒剂。

[0410] 103.根据实施方案102所述的方法,其中所述药剂是胆碱酯酶抑制剂。

[0411] 104.根据实施方案103所述的方法,其中所述胆碱酯酶抑制剂选自由加兰他敏、多奈哌齐、卡巴拉汀和他克林组成的组。

[0412] 105.根据实施方案102所述的方法,其中所述药剂是NMDA受体拮抗剂。

[0413] 106.根据实施方案105所述的方法,其中所述NMDA受体拮抗剂是美金刚胺或其盐。

[0414] 107.根据实施方案102所述的方法,其中所述药剂是特异性结合标靶的治疗剂且所述标靶选自由以下所组成的组:β分泌酶、tau、早老素、淀粉样前体蛋白或其部分、淀粉样β肽或其低聚物或原纤维、死亡受体6(DR6)、晚期糖基化终产物的受体(RAGE)、帕金蛋白和亨廷顿蛋白。

[0415] 108.根据实施方案102所述的方法,其中所述药剂是单胺耗竭剂,任选为丁苯喹嗪。

[0416] 109.根据实施方案102所述的方法,其中所述药剂是选自由以下所组成的组的抗胆碱能抗帕金森病剂:普环啶、苯海拉明、三己芬迪、苯托品、比哌立登和苯海索。

[0417] 110.根据实施方案102所述的方法,其中所述药剂是选自由以下所组成的组的多巴胺能抗帕金森病剂:恩他卡朋、司来吉兰、普拉克索、溴隐亭、罗替戈汀、司来吉兰、罗匹尼罗、雷沙吉兰、阿朴吗啡、卡比多巴、左旋多巴、培高利特、托卡朋和金刚烷胺。

[0418] 111.根据实施方案102所述的方法,其中所述药剂是选自由非类固醇类抗炎药和吲哚美辛组成的组的抗炎剂。

[0419] 112.根据实施方案102所述的方法,其中所述药剂是选自由雌激素、孕酮和亮丙瑞林组成的组的激素。

[0420] 113.根据实施方案102所述的方法,其中所述药剂是选自由叶酸和烟酰胺组成的组的维生素。

[0421] 114.根据实施方案102所述的方法,其中所述药剂是高牛磺酸,其为3-氨基丙磺酸或3APS。

[0422] 115.根据实施方案102所述的方法,其中所述药剂是扎利罗登。

[0423] 116.根据实施方案102所述的方法,其中所述药剂是除克雷内治单抗以外的抗Aβ抗体。

[0424] 实施例

[0425] 实施例1--克雷内治单抗(人源化抗Aβ单克隆抗体)在治疗轻到中度阿尔茨海默氏病中的临床研究

[0426] 研究设计和目标

[0427] 使用安慰剂对照实施随机化、双盲II期试验以评估人源化单克隆抗淀粉样蛋白β(“Aβ”)抗体克雷内治单抗在被诊断患有轻到中度阿尔茨海默氏病(AD)的患者中的作用。这项研究中包括的患者在筛选时年龄介于50与80之间,且根据NINCDS-ADRDA准则诊断可能患有AD,其中:简易智力状态检查(MMSE)评分为18至26点,老年抑郁量表(Geriatric Depression Scale)(GDS-15)评分小于6,完成6年教育(或良好的工作经历,无智力障碍或其它广泛性发育障碍)。另外,对于接受同时AD治疗(乙酰胆碱酯酶抑制剂或美金刚)的患者来说,确认患者已服药达至少3个月且在随机化之前处于稳定剂量达至少2个月。至少50%

的招募患者为ApoE4阳性(携带至少一个ApoE4等位基因)。还允许招募同时接受一种或多种未排除的处方或非处方医药(例如非抗胆碱能抗抑郁药、非典型抗精神病药、非苯丙二氮呯抗焦虑剂、催眠剂、中枢作用的抗胆碱能抗组胺和中枢作用的抗胆碱能抗痉挛剂)的患者,前提是所施用剂量在随机化之前恒定达至少1个月,并保持同一剂量达研究持续时间。

[0428] 如果存在以下情况,则将个体从试验中排除:遭受严重或不稳定的医学病况,根据研究者或赞助者的意见将干扰患者完成研究评价的能力或相当于将需要机构或医药照护;有或存在潜在影响大脑的临床上明显的血管疾病;有严重的、临床上显著的中枢神经系统创伤病史(例如永久性神经缺损或脑结构性损害);在筛选之前4周内住院;先前经过克雷内治单抗或任何其它靶向Aβ的药剂治疗;或如果在生物疗法中治疗剂的5个半衰期或在筛选之前3个月的较长时间内接受利用任何生物疗法(除常规疫苗以外)的治疗。

[0429] 研究有三个时期,即,持续长达35天的筛选期、持续68周的治疗期(在本文中称为第1周、第2周等,直到第69周)和再持续16周的安全性随访期(在本文中称为第70周等,直到第85周)。治疗(或安慰剂)是经由静脉内输注施用。

[0430] 将患者招募到试验中,并以2:1(治疗组:安慰剂组)随机化比随机分配到两个组中的一个,治疗(即,克雷内治单抗)组和安慰剂组。在试验中招募249名MMSE评分为18至26的患者(归类为轻到中度AD),其中165名接受治疗剂且84名接受安慰剂。治疗组中的121名患者和安慰剂中的61名患者具有介于20与26之间的MMSE评分(归类为轻度AD)。在治疗组内,117名(或70.9%)为ApoE4阳性。在安慰剂组中,60名患者(或71.4%)为ApoE4阳性。参见图4A-B(以患者状况制表)。

[0431] 实施43天的安全性导入评价以测定15mg/kg静脉内剂量对10mg/kg静脉内剂量的安全性和耐受性并选择15mg/kg的剂量。在试验的两个组中的患者都是每4周接受盲性静脉内注射达68周;基于安全性导入的结果,治疗组中的患者接受15mg/kg,而安慰剂组中的患者接受安慰剂的静脉内注射。参见图5(方案示意图)。

[0432] 72周后针对以下评估患者:(a)ADAS-Cog12评分和CDR-SOB评分在第25周、第49周和第73周从试验开始时的基线评分的变化,以评估疾病进展的抑制和(b)克雷内治单抗与安慰剂相比的安全性和耐受性。为估计任一所测量变化的统计显著性,计算共变异分析、信赖区间和自基线的平均变化差异的最小平方估计。

[0433] 通过测量整个实验中治疗意外不良事件的频率和严重性评价克雷内治单抗的安全性和耐受性,尤其症状性或无症状ARIA-E(包括脑血管源性水肿)、症状性或无症状ARIA-H(包括脑微出血)和脑大量出血的情形。通过液体衰减反转恢复核磁共振成像(FLAIR MRI)评价在筛选期期间(在开始给药之前)或在治疗期期间(开始利用安慰剂或克雷内治单抗给药之后)脑血管源性脑水肿情况的存在和/或数量。参见例如Sperling等人,2011,Alzheimer's&Dementia 7:367-385。通过横向磁化松弛时间和额外非均匀退相梯度回波核磁共振成像(T2*加权GRE MRI)评价在筛选期期间(在开始给药之前)或在治疗期期间(开始利用安慰剂或克雷内治单抗给药之后)脑微出血的存在和/或数量。

[0434] 结果

[0435] 在73周的ADAS-Cog12测量证实接受克雷内治单抗的患者显示比接受安慰剂的患者的较小的疾病进展。如图6A-B中所示表和图7-8中所示的图表中所汇总,ADAS-Cog12评分的变化对于患有轻度AD的患者在治疗组中比在安慰剂组中小约24%(p=0.12),且对于患

有轻到中度AD的患者，在治疗组中比在安慰剂组中小约16％(p＝0.19)。还在治疗组对安慰剂组中的ApoE4阳性患者之间发现这种效应:ADAS-Cog12评分的增加(其中ADAS-Cog12评分的增加指示疾病进展)在接受克雷内治单抗的患者中相对于接受安慰剂的患者小24.4％(p＝0.08，未针对多重性调整)。参见图6A和图9。ApoE4阳性患者包括患有轻度和中度AD的患者。当将轻度和ApoE4阳性患者两者的结果汇总时，这种效应更明显:在治疗组中相对于安慰剂看到降低32.4％(p＝0.05，未针对多重性调整)。参见图6A和图10。治疗效果随招募时MMSE评分的增加而增加。如图6B中所示，MMSE评分越高，在治疗组中相对于安慰剂组ADAS-Cog12的减少百分比越大，其范围在MMSE介于18与26之间的患者的约16％至高达MMSE介于25与26之间的患者的49％。还参见图11。对于MMSE评分介于22与26之间的患者，在治疗组中与安慰剂相比ADAS-Cog12的减少百分比为约35％。

[0436] CDR-SOB的变化示出类似治疗效果趋势。如图12A中所示，对于MMSE在22与26的患者，在治疗组对安慰剂中看到CDR-SOB评分变化的19％减少，且在MMSE评分在25至26的患者中，这种效果甚至更显著，其中减少百分比为约63％(还参见图13)。图12B示出对于MMSE在22至26的患者，在查看记忆或判断与问题解决分量评分时，减少百分比分别为约42％和30％。

[0437] 研究进一步证实克雷内治单抗在以15mg/kg的剂量施用时不会增加ARIA型事件的发生率。在研究中，在接受克雷内治单抗的患者中观察到单一、无症状ARAI-E事件。ARAI-H事件的数量在治疗组与安慰剂组之间达成平衡。

[0438] 这些数据证实，当在患有轻到中度AD的患者，具体地说患有轻度AD和/或为ApoE4阳性的患者中以15mg/kg的剂量施用时，克雷内治单抗抑制疾病进展，而不会增加治疗意外不良事件(例如ARIA-E或ARIA-H)的发生率。

[0439] 实施例2--克雷内治单抗(人源化抗Aβ单克隆抗体)在治疗轻到中度阿尔茨海默氏病和评估对淀粉样蛋白负荷的影响的临床研究研究设计和目标

[0440] 使用安慰剂对照实施随机化、双盲II期试验以评估人源化单克隆抗淀粉样蛋白β(“Aβ”)抗体克雷内治单抗在被诊断患有轻到中度阿尔茨海默氏病(AD)的患者中的作用。这项研究中包括的患者在筛选时年龄介于50与80之间，且根据NINCDS-ADRDA准则诊断可能患有AD，其中:简易智力状态检查(MMSE)评分为18至26点，老年抑郁量表(Geriatric Depression Scale)(GDS-15)评分小于6，完成6年教育(或良好的工作经历，无智力障碍或其它广泛性发育障碍)。只招募在筛选时具有阳性florbetapir PET(“淀粉样蛋白阳性”)扫描的患者，这指示大脑淀粉样蛋白负荷增加在被诊断患有AD的患者所预计的范围内，如通过florbetapir-PET扫描所评价。另外，至少50％的招募患者为ApoE4阳性。

[0441] 将患者招募到试验中，并以2:1(治疗组:安慰剂组)随机化比随机分配到两个组中的一个，治疗(即，克雷内治单抗)组和安慰剂组。在试验的两个组中，52名患者都是每4周接受盲性静脉内注射达73周。在治疗组中，患者接受15mg/kg剂量的克雷内治单抗。根据以下将患者分层:ApoE4状态(载剂对非载剂)和MMSE评分。

[0442] 收集以下数据的变化:ADAS-Cog12、淀粉样蛋白负担(如使用florbetapir-PET所测量)和脑脊髓液(CSF)中的Aβ含量。在筛选时、12个月和18个月随访时使用florbetapir 10mCi获得Florbetapir PET扫描，其中50-min吸收期和30min发射扫描。将来自6X5分钟框(或在无动态性能的扫描仪上1X15分钟框)的影像正规化为标准空间，其中使用模板来自若

干感兴趣区域(ROI)提取平均信号。使用基线T1加权MRI扫描来精修模板ROI的容积。使用小脑皮质或皮质下白质作为参考区域实施分析。在筛选时和在第18个月施用之前收集CSF。测量CSF Aβ、tau和p-tau(181)。使用重复测量的ANCOVA或混合模型用于在研究终点的治疗差异的统计分析。

[0443] 患者特性、不良事件和PET扫描、MRI扫描和CSF取样的时间示于图14A-B中。

[0444] 结果。在治疗期结束时ADAS-Cog12测量值证实，接受克雷内治单抗比接受安慰剂的患者示出更小的疾病进展。在初始MMSE评分介于20与26之间的患者中观察到认知衰退减少54.3%(p＝0.2)。与此示出疾病进展的观察结果相一致，在利用克雷内治单抗治疗的患者对接受安慰剂的患者中，通过PET分析(利用皮质下白质参考区域)也观察到淀粉样蛋白沉积的累积降低。参见图15A。此外，在治疗组中检测到Aβ的脑脊髓液浓度增加，这与靶标由克雷内治单抗结合相一致。参见图15B。在每2周利用300mg皮下施用克雷内治单抗治疗的患者中对接受安慰剂的患者检测到Aβ的脑脊髓液浓度的类似增加。

[0445] 这些数据表明克雷内治单抗结合其靶标淀粉样蛋白β，且在患有轻到中度AD的患者，具体地说患有轻度AD的患者中(包括具有通常被诊断患有AD的患者中所见的大脑淀粉样蛋白负担的患者中)以15mg/kg的剂量施用时抑制疾病进展。

[0446] 尽管已经通过说明和实施例描述了上述发明的一些细节，但这些描述和实施例不应该被视为限制本发明的范围。本文所引用的所有专利申请和出版物以及科学文献的公开内容明确地通过引用整体并入以供所有目的使用。

[0447] 序列表关键内容

[0448]

| **SEQ ID NO:** | ***序列*** |
|---|---|
| 1 | 人类Aβ1-42氨基酸序列：DAEFRHDSGYEVHHQKLVFFAEDVGSNKGAIIGLMVGGVVIA |

[0449]

| | |
|---|---|
| 2 | 克雷内治单抗 HVR-H1 氨基酸序列：GFTFSSYGMS |
| 3 | 克雷内治单抗 HVR-H2 氨基酸序列：SINSNGGSTYYPDSVK |
| 4 | 克雷内治单抗 HVR-H3 氨基酸序列：GDY |
| 5 | 克雷内治单抗重链氨基酸序列(HVR 区标记为粗体)：<br>EVQLVESGGGLVQPGGSLRLSCAAS**GFTFSSYGMS**WV<br>RQAPGKGLELVA**SINSNGGSTYYPDSVK**GRFTISRDNA<br>KNSLYLQMNSLRAEDTAVYYCAS**GDY**WGQGTTVTVSS<br>ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTV<br>SWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGT<br>KTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLG<br>GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQF<br>NWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQ<br>DWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVY<br>TLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP<br>ENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSC<br>SVMHEALHNHYTQKSLSLSLG |
| 6 | 克雷内治单抗 HVR-L1 氨基酸序列：RSSQSLVYSNGDTYLH |
| 7 | 克雷内治单抗 HVR-L2 氨基酸序列： KVSNRFS |
| 8 | 克雷内治单抗 HVR-L3 氨基酸序列：SQSTHVPWT |
| 9 | 克雷内治单抗轻链氨基酸序列(HVR 区标记为粗体和下划线)：<br>DIVMTQSPLSLPVTPGEPASISC**RSSQSLVYSNGDTYLH**<br>WYLQKPGQSPQLLIY**KVSNRFS**GVPDRFSGSGSGTDFT<br>LKISRVEAEDVGVYYC**SQSTHVPWT**FGQGTKVEIKRT<br>VAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ<br>WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKAD<br>YEKHKVYACEVTHQGLSSPVTKSFNRGEC |

序列表

<110> 健泰科生物技术公司

<120> 治疗阿尔茨海默氏病的方法

<130> P5696R1-WO

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<213> 智人

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

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

Ala Ser Ile Asn Ser Asn Gly Gly Ser Thr Tyr Tyr Pro 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 Val Tyr Tyr Cys
85 90 95

Ala Ser Gly Asp Tyr Trp Gly Gln Gly Thr Thr Val Thr Val Ser Ser
100 105 110

Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Cys Ser Arg
115 120 125

Ser Thr Ser Glu Ser Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr
130 135 140

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

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

Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Lys Thr
180 185 190

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

Arg Val Glu Ser Lys Tyr Gly Pro Pro Cys Pro Pro Cys Pro Ala Pro
210 215 220

Glu Phe Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
225 230 235 240

Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
245 250 255

Asp Val Ser Gln Glu Asp Pro Glu Val Gln Phe Asn Trp Tyr Val Asp
260 265 270

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

[0003] Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
290 295 300

Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Gly Leu
305 310 315 320

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

Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Gln Glu Glu Met Thr Lys
340 345 350

Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
355 360 365

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

Thr Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser
385 390 395 400

Arg Leu Thr Val Asp Lys Ser Arg Trp Gln Glu Gly Asn Val Phe Ser
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Cys Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser
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Leu Ser Leu Ser Leu Gly
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[0004]

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Glu Pro Ala Ser Ile Ser Cys Arg Ser Ser Gln Ser Leu Val Tyr Ser
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Asn Gly Asp Thr Tyr Leu His Trp Tyr Leu Gln Lys Pro Gly Gln Ser
35 40 45

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

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

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

Thr His Val Pro Trp Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys
100 105 110

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

[0005]

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

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

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

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

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

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

SEQ ID NO:1

1 DAEFRHDSGY EVHHQKLVFF AEDVGSNKGA IIGLMVGGVV IA

图1

HVR-HI (SEQ ID NO:2): GFTFSSYGMS
HVR-H2 (SEQ ID NO:3): SINSNGGSTYYPDSVK
HVR-H3 (SEQ ID NO:4): GDY

HVR-L1 (SEQ ID NO:6): RSSQSLVYSNGDTYLH
HVR-L2 (SEQ ID NO:7): KVSNRFS
HVR-L3 (SEQ ID NO:8): SQSTHVPWT

图2

HC 序列 (SEQ ID NO:5)

```
  1 EVQLVESGGG LVQPGGSLRL SCAASGFTFS SYGMSWVRQA PGKGLELVAS
 51 INSNGGSTYY PDSVKGRFTI SRDNAKNSLY LQMNSLRAED TAVYYCASGD
101 YWGQGTTVTV SSASTKGPSV FPLAPCSRST SESTAALGCL VKDYFPEPVT
151 VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT KTYTCNVDHK
201 PSNTKVDKRV ESKYGPPCPP CPAPEFLGGP SVFLFPPKPK DTLMISRTPE
251 VTCVVVDVSQ EDPEVQFNWY VDGVEVHNAK TKPREEQFNS TYRVVSVLTV
301 LHQDWLNGKE YKCKVSNKGL PSSIEKTISK AKGQPREPQV YTLPPSQEEM
351 TKNQVSLTCL VKGFYPSDIA VEWESNGQPE NNYKTTPPVL DSDGSFFLYS
401 RLTVDKSRWQ EGNVFSCSVM HEALHNHYTQ KSLSLSLG
```

LC 序列 (SEQ ID NO:9)

```
  1 DIVMTQSPLS LPVTPGEPAS ISCRSSQSLV YSNGDTYLHW YLQKPGQSPQ
 51 LLIYKVSNRF SGVPDRFSGS GSGTDFTLKI SRVEAEDVGV YYCSQSTHVP
101 WTFGQGTKVE IKRTVAAPSV FIFPPSDEQL KSGTASVVCL LNNFYPREAK
151 VQWKVDNALQ SGNSQESVTE QDSKDSTYSL SSTLTLSKAD YEKHKVYACE
201 VTHQGLSSPV TKSFNRGEC
```

图3

| | IV (N=249) | |
|---|---|---|
| | 安慰剂 (N=84) | 克雷内治单抗 (N=165) |
| AD的状态-Conmed使用 | | |
| n | 84 | 165 |
| 无 | 11 (13.1%) | 19 (11.5%) |
| 仅AchEI | 50 (59.5%) | 96 (58.2%) |
| 仅美金刚胺 | 2 (2.4%) | 8 (4.8%) |
| AchEI和美金刚胺 | 21 (25.0%) | 42 (25.5%) |
| ADAS-Cog12: 轻到中度 | | |
| | 84 | 163 |
| 平均值 (SD) | 27.08 (7.52) | 28.87 (9.17%) |
| 中位数 | 26.50 | 28.33 |
| 最小值-最大值 | 12.7 - 52.0 | 7.3 - 55.0 |

图4A

| | IV (N=249) | |
|---|---|---|
| | 安慰剂 (N=84) | 克雷内治单抗 (N=165) |
| 实际 Apoe4 结果 | | |
| n | 84 | 165 |
| E2/E3 | 2 (2.4%) | 6 (3.6%) |
| E2/E4 | 4 (4.8%) | 6 (3.6%) |
| E3/E3 | 22 (26.2%) | 42 (25.5%) |
| E3/E4 | 39 (46.4%) | 76 (46.1%) |
| E4/E4 | 17 (20.2%) | 35 (21.2%) |
| APOE4 载剂 | | |
| n | 84 | 165 |
| 阴性 | 24 (28.6%) | 48 (29.1%) |
| 阳性 | 60 (71.4%) | 117 (70.9%) |
| MMSE 分布 | | |
| n | 84 | 165 |
| 轻度 (20-26) | 61 (72.6%) | 121 (73.3%) |
| 中度 (18-19) | 23 (27.4%) | 44 (26.7%) |
| 筛选时的 MMSE分数 | | |
| n | 84 | 165 |
| 平均值 (50) | 21.60 (2.51%) | 21.85 (2.72%) |
| 中位数 | 22.00 | 22.00 |
| 最小值-最大值 | 18.0 - 26.0 | 16.0 - 26.0 |

图4B

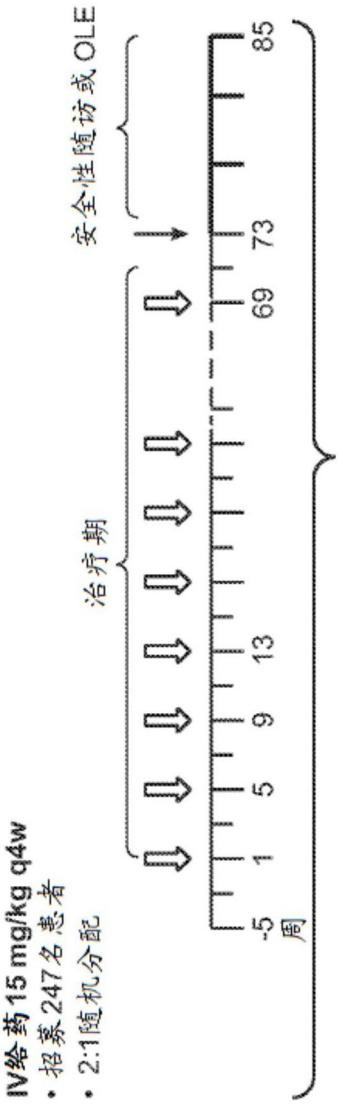



图5

**IV定群ADAS-Cog12：基于观察到的病例数据的第73周MMRM结果的概要**

| 群体 | 第73周的N | | 安慰剂LS平均(SE)变化分数 | 克雷内治单抗LS平均(SE)变化分数 | 差异 | | | 减少 % |
|---|---|---|---|---|---|---|---|---|
| | 安慰剂 | 克雷内治单抗 | | | LS平均值(SE) | 80% CI | P值 | |
| M2M | 64 | 122 | 10.85 (1.10) | 9.07 (0.81) | 1.78 (1.35) | 0.04, 3.51 | 0.19 | 16% |
| ApoE4 阴性 | 21 | 32 | 9.34 (2.04) | 9.83 (1.59) | -0.50 (2.59) | -3.85, 2.86 | 0.849* | -5% |
| ApoE4 阳性 | 43 | 90 | 11.19 (1.29) | 8.46 (0.91) | 2.73 (1.58) | 0.70, 4.77 | 0.086* | 24% |
| 轻度 | 47 | 93 | 10.09 (1.22) | 7.85 (0.90) | 2.24 (1.47) | 0.36, 4.13 | 0.128* | 22% |
| 轻度和 ApoE4 阳性 | 31 | 68 | 9.89 (1.37) | 6.68 (0.97) | 3.21 (1.64) | 1.08, 5.33 | 0.054* | 32% |
| 中度 | 17 | 29 | 13.83 (2.38) | 13.68 (1.89) | 0.16 (2.99) | -3.74, 4.05 | 0.959* | 1% |

*未调整 p 值的多重性

图6A

IV定群ADAS-Cog12：基于观察到的病例数据的第73周MMRM结果的概要

| MMSE范围 (MITT的%) | 第73周的N | | 安慰剂LS平均(SE)变化分数 | 克雷内治单抗平均(SE)变化分数 | 差异 | | | 减少% | ES (SD) |
|---|---|---|---|---|---|---|---|---|---|
| | 安慰剂 | 克雷内治单抗 | | | LS平均值(SE) | 80% CI | P值 | | |
| 18-26 (100%) | 64 | 122 | 10.56 (1.09) | 8.79 (0.79) | 1.78 (1.35) | 0.04, 3.51 | 0.190 | 16.8% | 0.20 (9.08) |
| 19-26 (87%) | 56 | 105 | 10.18 (1.15) | 8.07 (0.84) | 2.12 (1.42) | 0.29, 3.95 | 0.139 | 20.80% | 0.24 (8.89) |
| 20-26 (74%) | 47 | 93 | 9.43 (1.20) | 7.18 (0.85) | 2.24 (1.47) | 0.36, 4.13 | 0.128 | 23.8% | 0.27 (8.44) |
| 21-26 (64%) | 39 | 83 | 9.22 (1.30) | 6.96 (0.90) | 2.26 (1.58) | 0.22, 4.30 | 0.157 | 24.5% | 0.27 (8.40) |
| 22-26 (54%) | 33 | 70 | 9.70 (1.33) | 6.26 (0.91) | 3.44 (1.61) | 1.36, 5.52 | 0.036 | 35.4% | 0.44 (7.80) |
| 23-26 (43%) | 24 | 60 | 7.92 (1.44) | 5.51 (0.91) | 2.40 (1.70) | 0.20, 4.60 | 0.163 | 30.30% | 0.33 (7.18) |
| 24-26 (32%) | 16 | 45 | 7.41 (1.77) | 4.58 (1.06) | 2.83 (2.07) | 0.15, 5.51 | 0.176 | 38.20% | 0.39 (7.25) |
| 25-26 (21%) | 11 | 30 | 6.88 (2.13) | 3.51 (1.31) | 3.37 (2.50) | 0.11, 6.63 | 0.185 | 49.00% | 0.47 (7.21) |

图6B

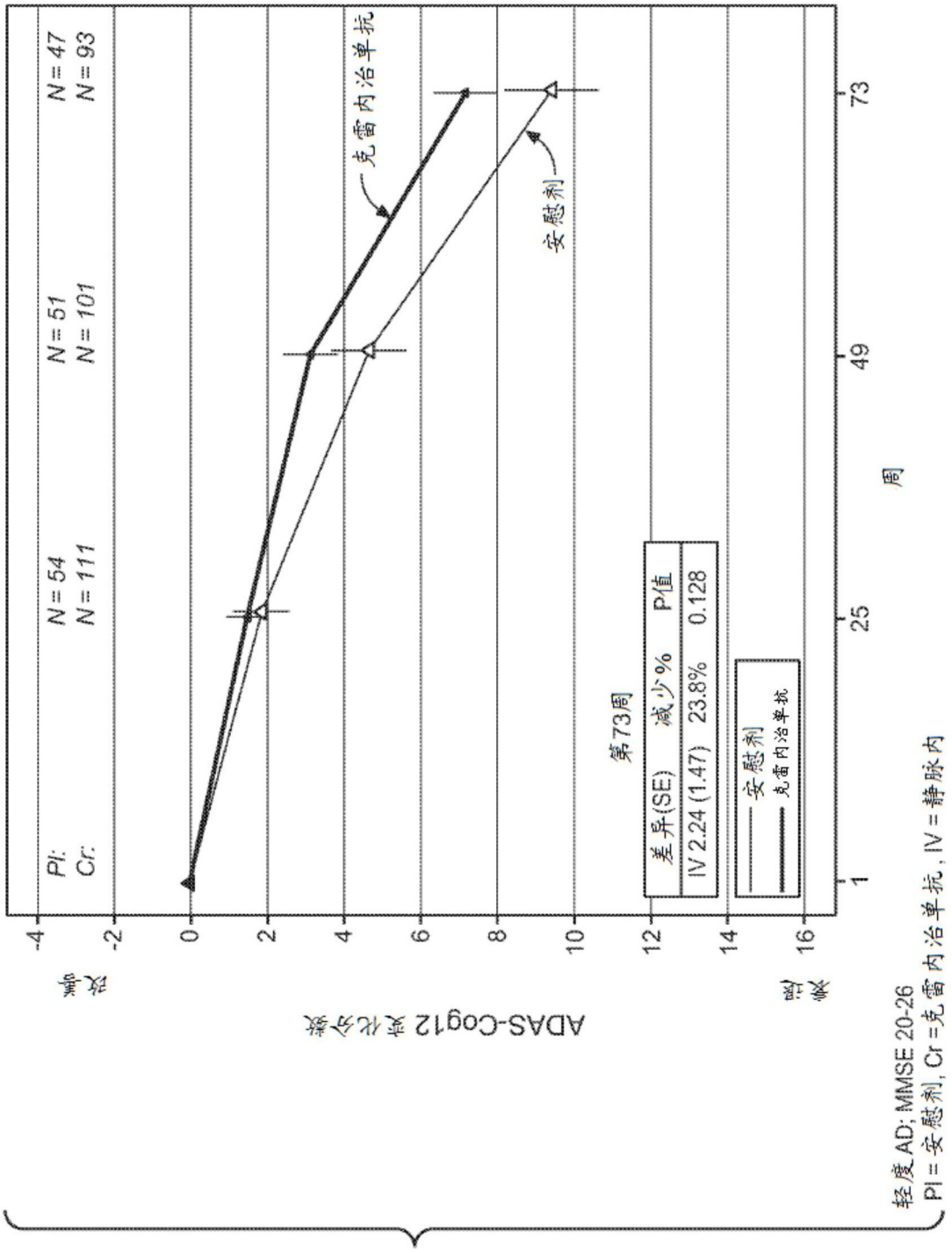

ADAS-Cog12 变化分数
改善
恶化
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
周
PI:
Cr:
N = 54
N = 111
N = 51
N = 101
N = 47
N = 93
克雷内治单抗
安慰剂
第73周
差异(SE) 减少% P值
IV 2.24 (1.47) 23.8% 0.128
安慰剂
克雷内治单抗
轻度AD; MMSE 20-26
PI = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图7

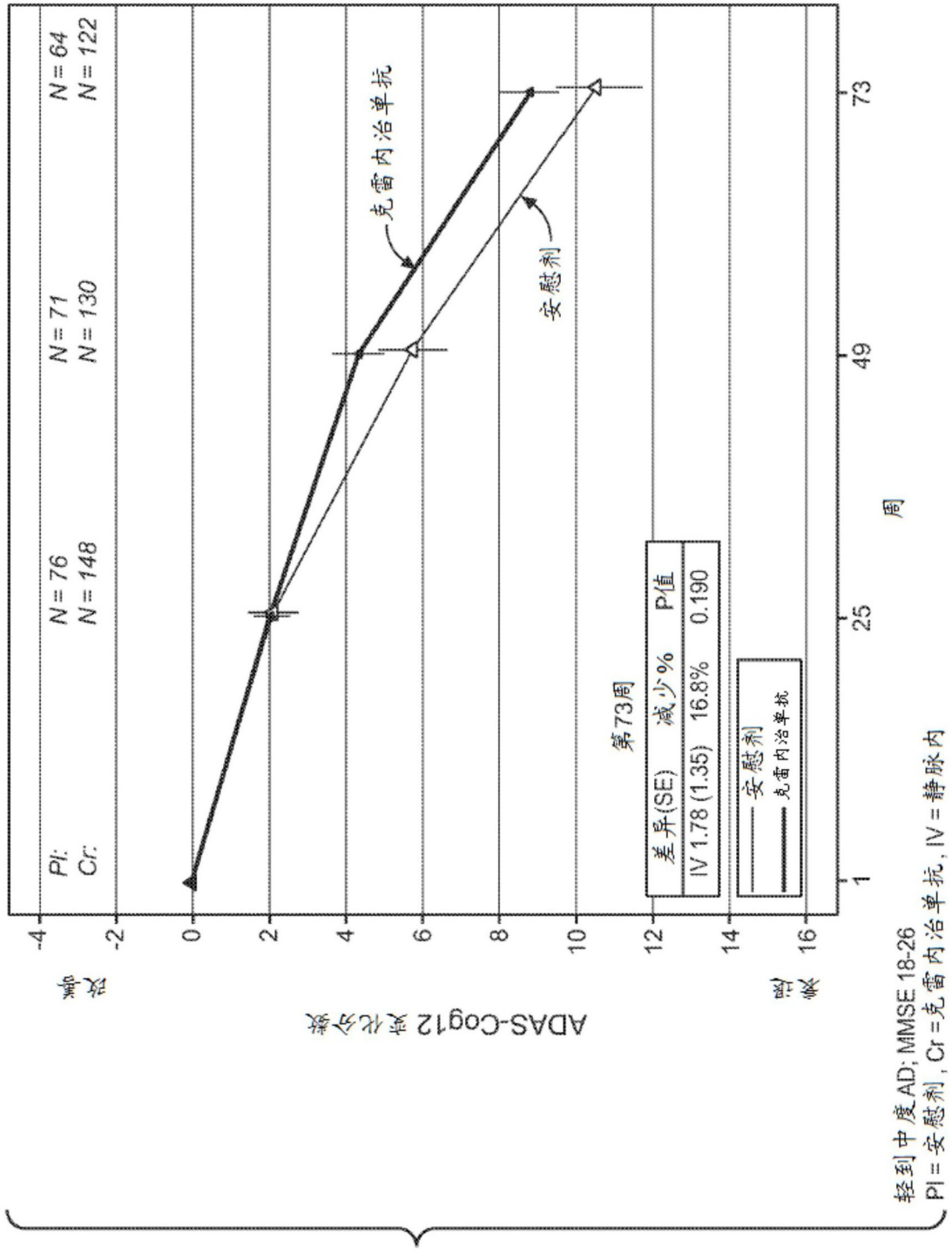

ADAS-Cog12 变化分数
改善
恶化
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
周
克雷内治单抗
安慰剂
Pl: N = 76 N = 71 N = 64
Cr: N = 148 N = 130 N = 122
第73周
差异(SE) 减少% P值
IV 1.78 (1.35) 16.8% 0.190
安慰剂
克雷内治单抗
轻到中度AD; MMSE 18-26
Pl = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图8

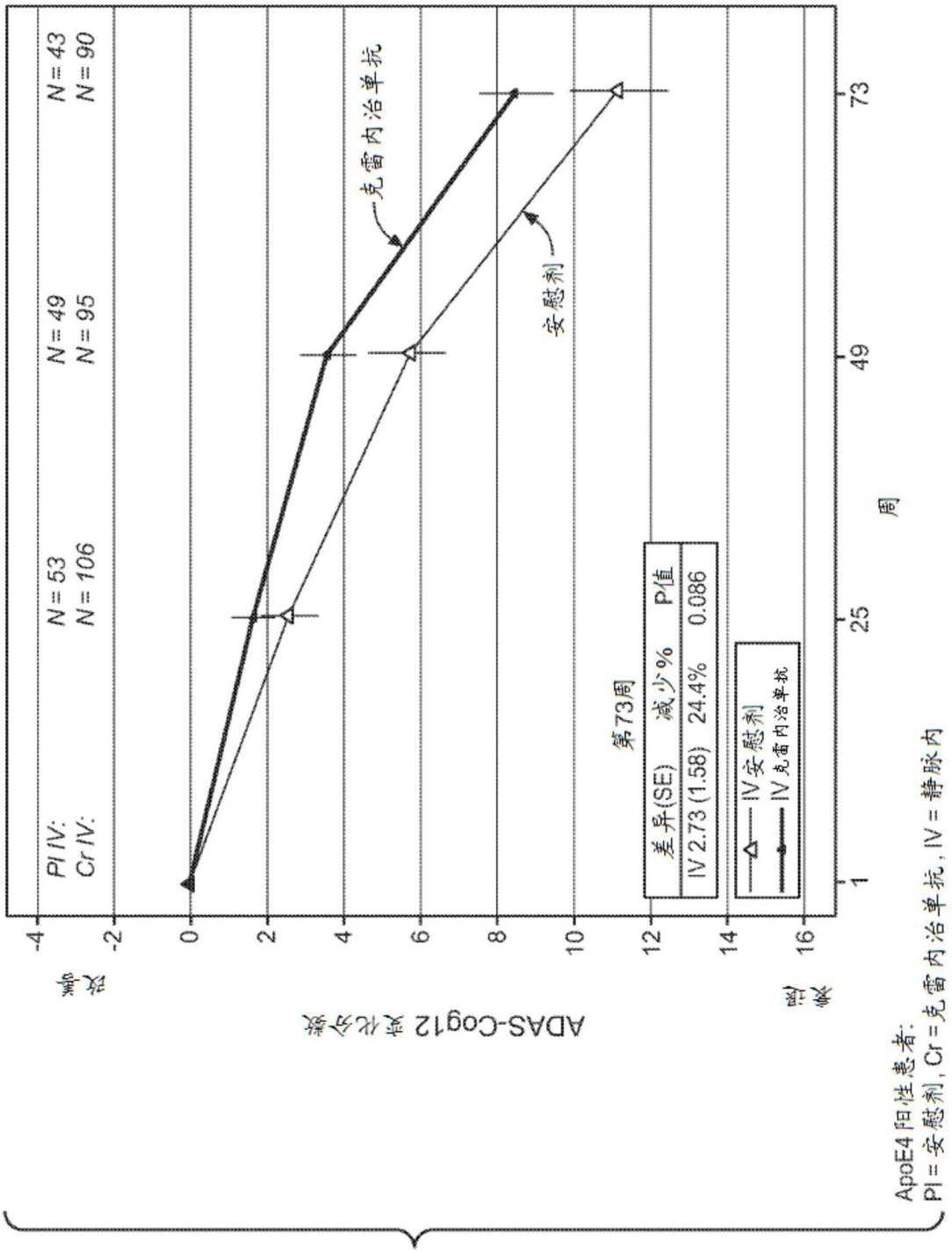

ADAS-Cog12 变化分数
改善
恶化
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
周
克雷内治单抗
安慰剂
PI IV: N = 53 N = 49 N = 43
Cr IV: N = 106 N = 95 N = 90
第73周
差异(SE) 减少% P值
IV 2.73 (1.58) 24.4% 0.086
IV安慰剂
IV克雷内治单抗
ApoE4 阳性患者:
PI = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图9

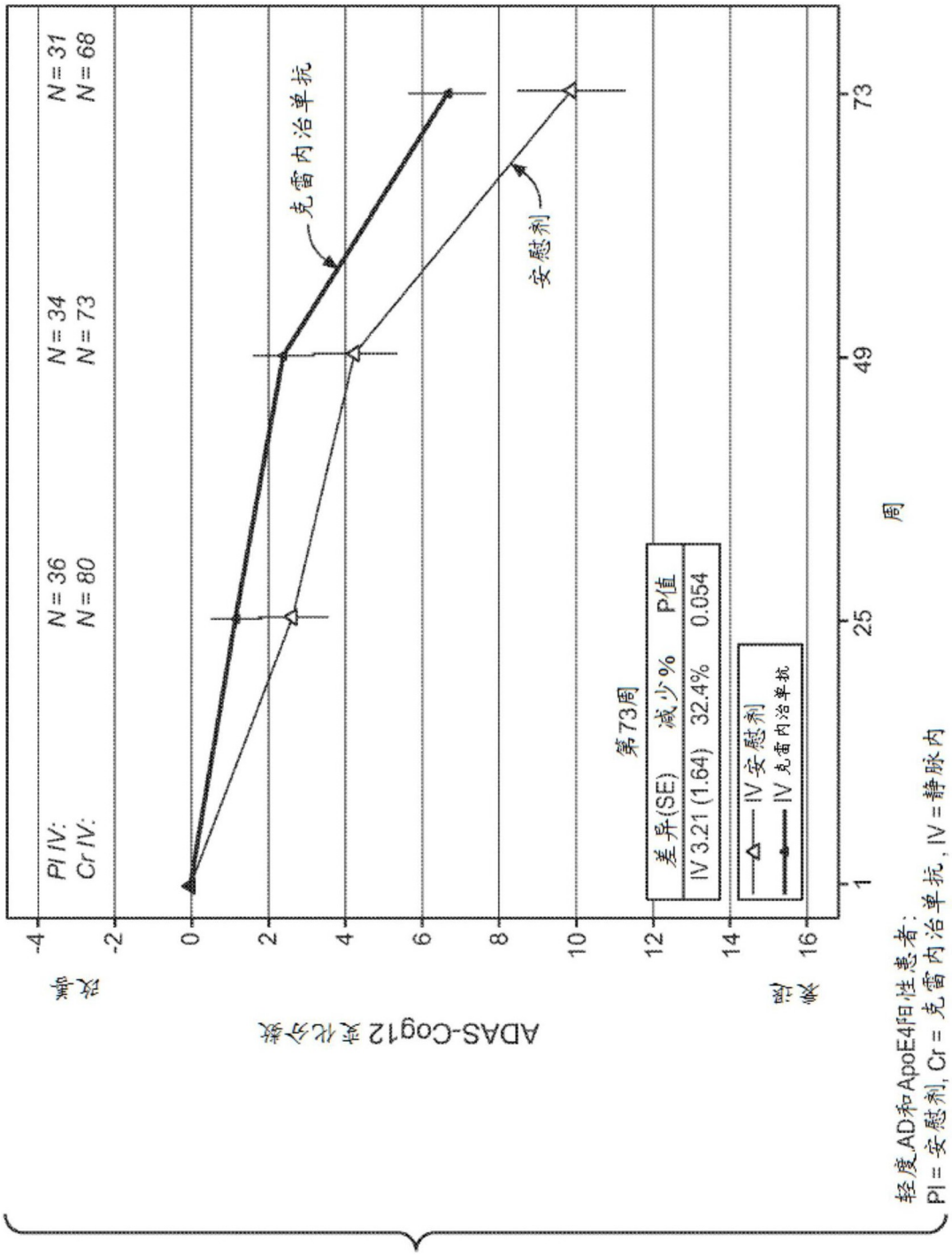

ADAS-Cog12 变化分数
改善
恶化
周
克雷内治单抗
安慰剂
Pl IV: N = 36 N = 80
Cr IV: N = 34 N = 73
N = 31 N = 68
第73周
差异(SE) 减少% P值
IV 3.21 (1.64) 32.4% 0.054
IV 安慰剂
IV 克雷内治单抗
轻度AD和ApoE4阳性患者;
Pl = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图10

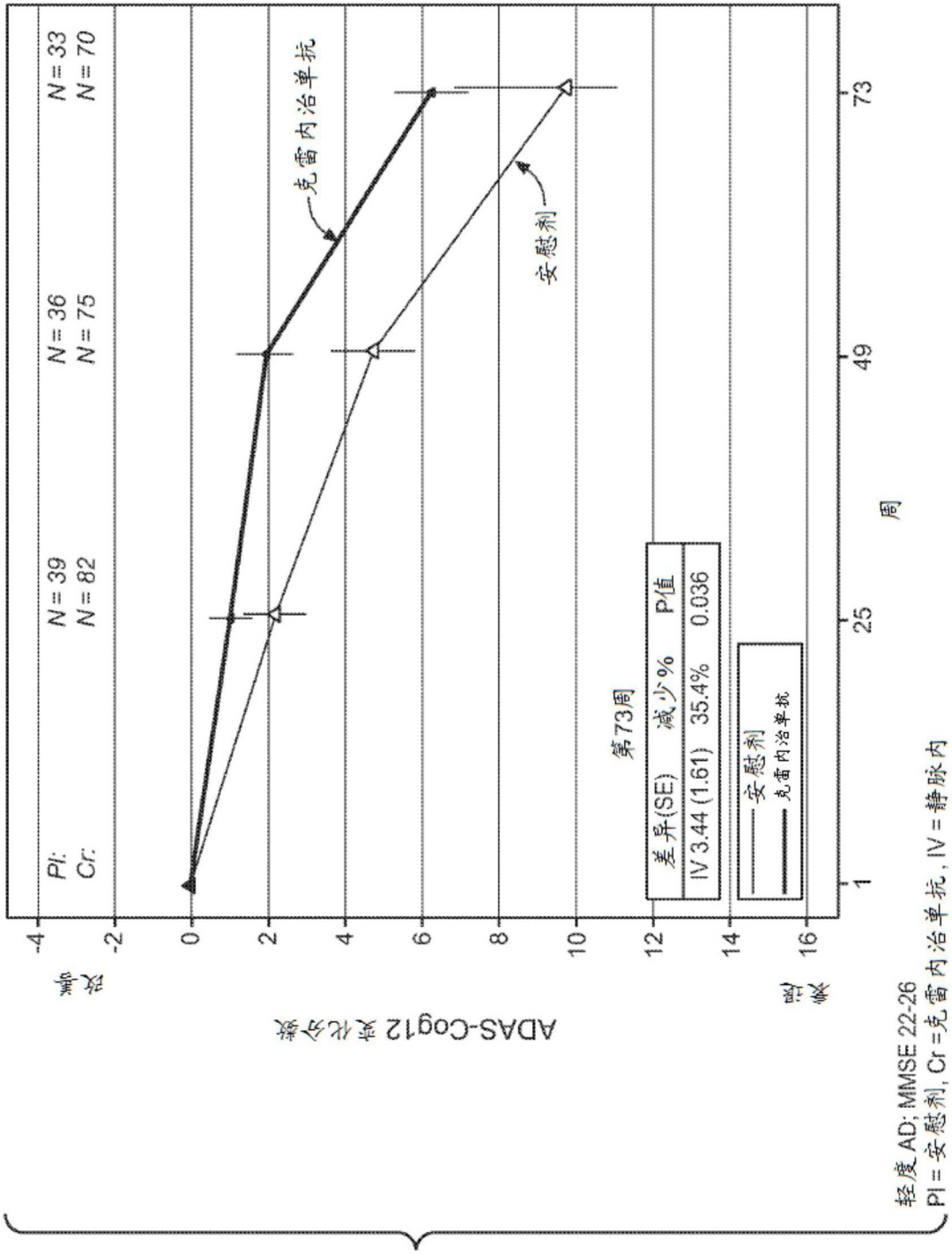

ADAS-Cog12 变化分数
改善
恶化
-4
-2
0
2
4
6
8
10
12
14
16
1
25
49
73
周
N = 39
N = 82
N = 36
N = 75
N = 33
N = 70
Pl:
Cr:
克雷内治单抗
安慰剂
第73周
差异(SE) 减少% P值
IV 3.44 (1.61) 35.4% 0.036
安慰剂
克雷内治单抗
轻度 AD; MMSE 22-26
Pl = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图11

IV定群 CDR-SOB:基于观察到的病例数据的第73周 MMRM结果

| MMSE范围 (MITT的%) | 第73周的N | | 安慰剂LS平均(SE)变化分数 | 克雷内治单抗LS平均(SE)变化分数 | 差异 | | | 减少% | ES (SD) |
|---|---|---|---|---|---|---|---|---|---|
| | 安慰剂 | 克雷内治单抗 | | | LS平均值(SE) | 80% CI | P值 | | |
| 18-26 (100%) | 67 | 126 | 2.57 (0.35) | 2.48 (0.25) | 0.09 (0.43) | -0.46, 0.64 | 0.837 | 3.4% | 0.03 (2.94) |
| 19-26 (86%) | 58 | 108 | 2.65 (0.38) | 2.43 (0.28) | 0.22 (0.47) | -0.39, 0.83 | 0.641 | 8.30% | 0.07 (3.02) |
| 20-26 (74%) | 48 | 96 | 2.18 (0.40) | 2.21 (0.28) | -0.02 (0.49) | -0.66, 0.61 | 0.964 | -1.0% | -0.01 (2.91) |
| 21-26 (64%) | 40 | 85 | 2.26 (0.45) | 2.16 (0.31) | 0.10 (0.54) | -0.60, 0.81 | 0.848 | 4.6% | 0.04 (2.98) |
| 22-26 (54%) | 34 | 71 | 2.24 (0.45) | 1.80 (0.31) | 0.44 (0.55) | -0.27, 1.14 | 0.423 | 19.6% | 0.16 (2.75) |
| 23-26 (42%) | 24 | 60 | 1.88 (0.45) | 1.48 (0.28) | 0.40 (0.53) | -0.28, 1.08 | 0.449 | 21.40% | 0.18 (2.25) |
| 24-26 (31%) | 16 | 45 | 1.87 (0.45) | 1.02 (0.27) | 0.85 (0.52) | 0.16, 1.53 | 0.114 | 45.40% | 0.46 (1.85) |
| 25-26 (20%) | 11 | 30 | 1.95 (0.44) | 0.71 (0.27) | 1.24 (0.52) | 0.56, 1.92 | 0.022 | 63.60% | 0.83 (1.50) |

图12A

| MMSE | N (Plc) | N (Cre) | CDR-SOB 总和 Δ (SE) | Δ % | ES (SD) | P | CDR-SOB 判断和问题解决 Δ (SE) | Δ % | P | CDR-SOB 记忆 Δ (SE) | Δ % | P |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18-26 | 67 | 126 | 0.09 (0.43) | 3.4% | 0.03 (2.94) | 0.837 | 0.02 (0.08) | 5.7% | 0.790 | 0.08 (0.08) | 18.2% | 0.311 |
| 20-26 | 48 | 96 | -0.02 (0.49) | -1.0% | 0.01 (2.91) | 0.964 | 0.06 (0.09) | 16.1% | 0.517 | 0.09 (0.09) | 21.7% | 0.346 |
| 22-26 | 34 | 71 | 0.44 (0.55) | 19.6% | 0.16 (2.75) | 0.423 | 0.12 (0.10) | 29.9% | 0.236 | 0.16 (0.10) | 42.7% | 0.124 |

图12B

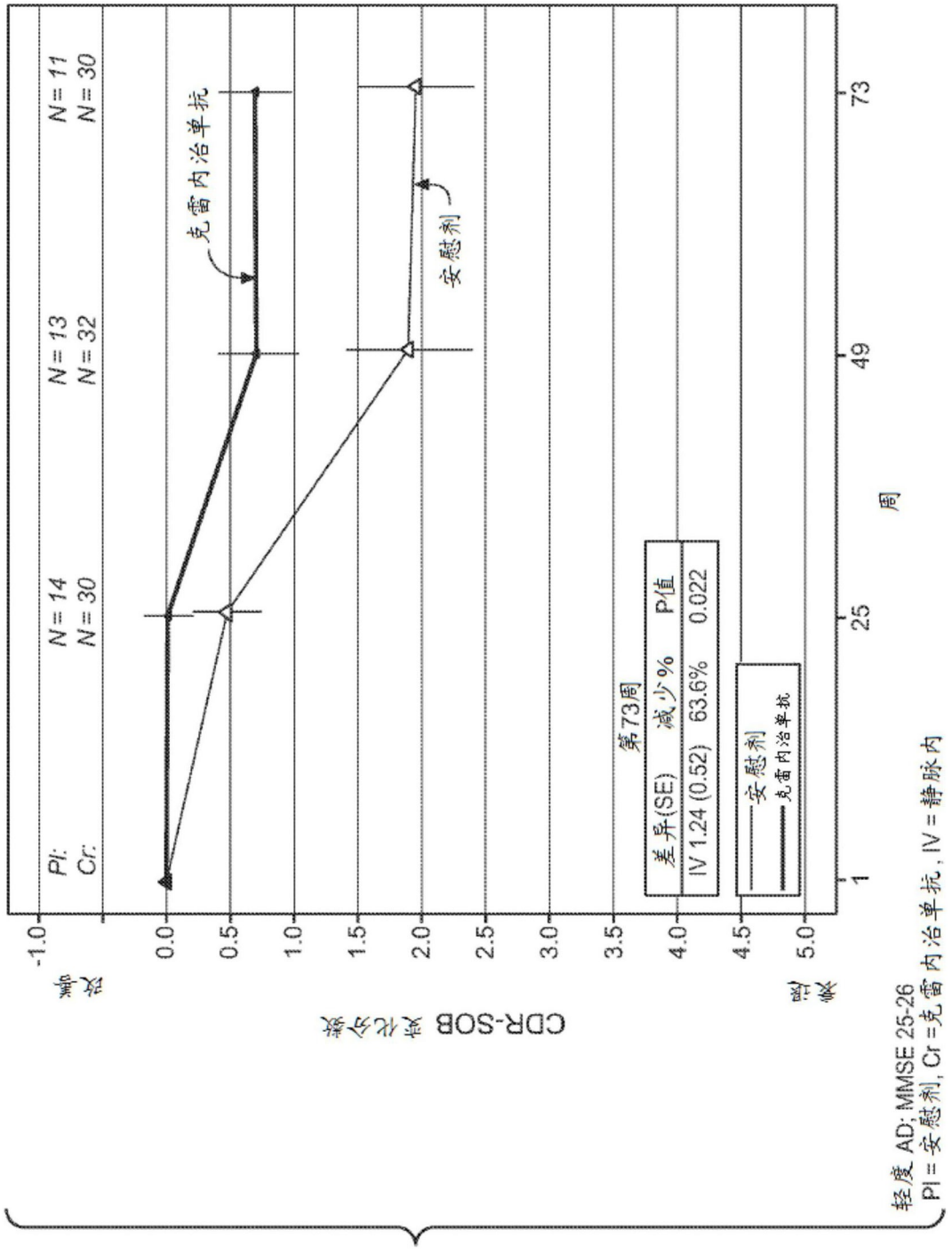
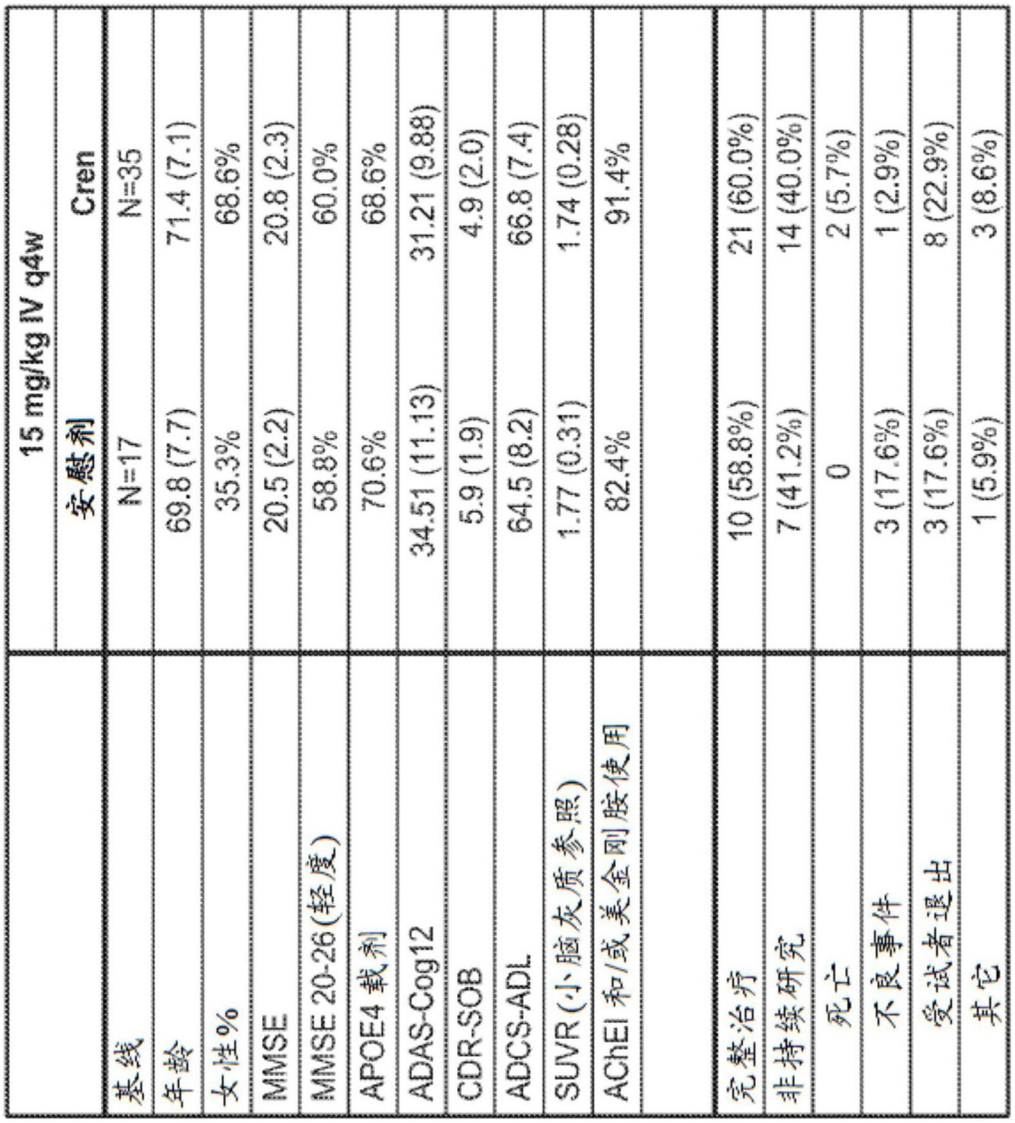

CDR-SOB 变化分数
改善
恶化
-1.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
1
25
49
73
周
N = 14
N = 30
N = 13
N = 32
N = 11
N = 30
PI:
Cr:
克雷内治单抗
安慰剂
第73周
差异(SE) 减少% P值
IV 1.24 (0.52) 63.6% 0.022
安慰剂
克雷内治单抗
轻度 AD; MMSE 25-26
PI = 安慰剂, Cr = 克雷内治单抗, IV = 静脉内

图13



| | 15 mg/kg IV q4w | |
|---|---|---|
| | 安慰剂 | Cren |
| 基线 | N=17 | N=35 |
| 年龄 | 69.8 (7.7) | 71.4 (7.1) |
| 女性% | 35.3% | 68.6% |
| MMSE | 20.5 (2.2) | 20.8 (2.3) |
| MMSE 20-26(轻度) | 58.8% | 60.0% |
| APOE4 载剂 | 70.6% | 68.6% |
| ADAS-Cog12 | 34.51 (11.13) | 31.21 (9.88) |
| CDR-SOB | 5.9 (1.9) | 4.9 (2.0) |
| ADCS-ADL | 64.5 (8.2) | 66.8 (7.4) |
| SUVR(小脑灰质参照) | 1.77 (0.31) | 1.74 (0.28) |
| AChEI 和/或美金刚胺使用 | 82.4% | 91.4% |
| | | |
| 完整治疗 | 10 (58.8%) | 21 (60.0%) |
| 非持续研究 | 7 (41.2%) | 14 (40.0%) |
| 死亡 | 0 | 2 (5.7%) |
| 不良事件 | 3 (17.6%) | 1 (2.9%) |
| 受试者退出 | 3 (17.6%) | 8 (22.9%) |
| 其它 | 1 (5.9%) | 3 (8.6%) |

图14A

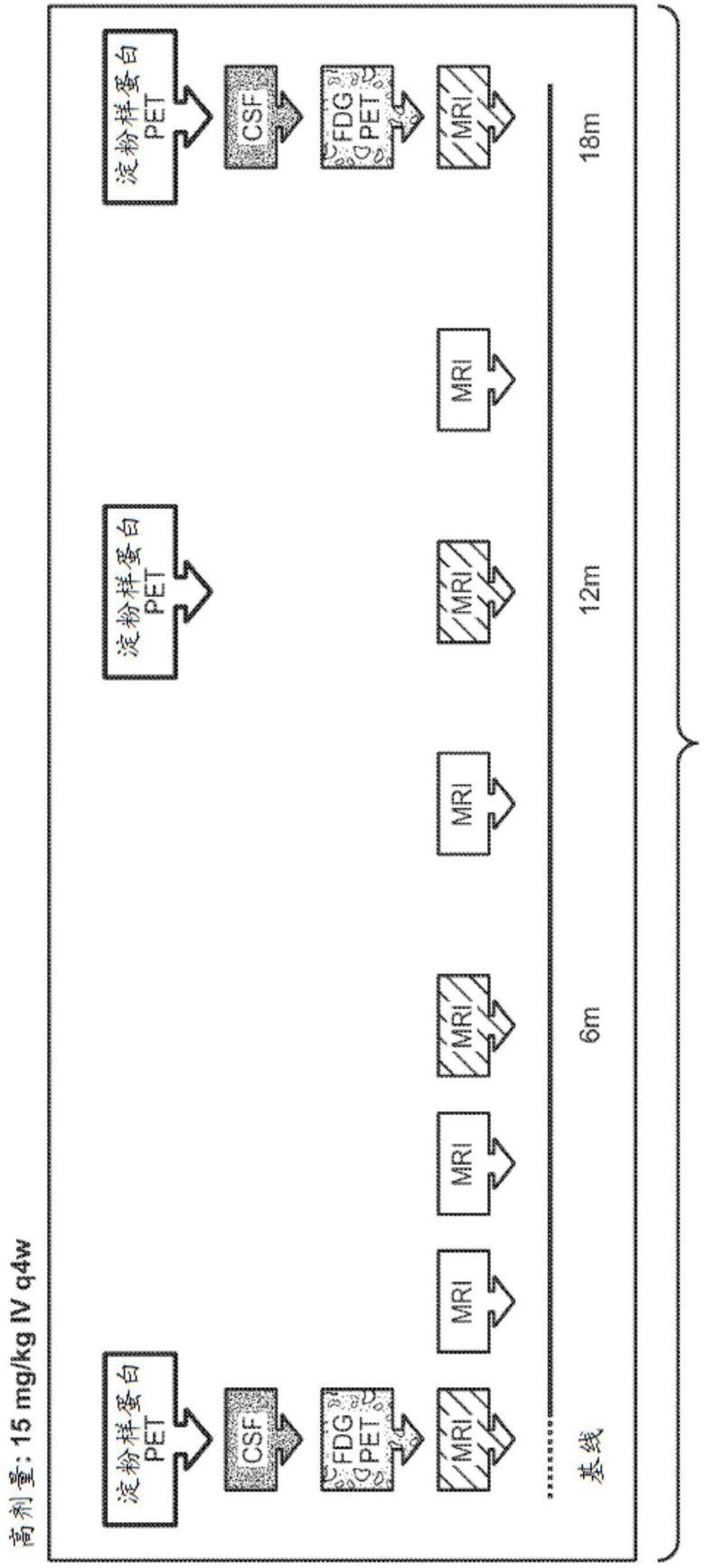



图14B

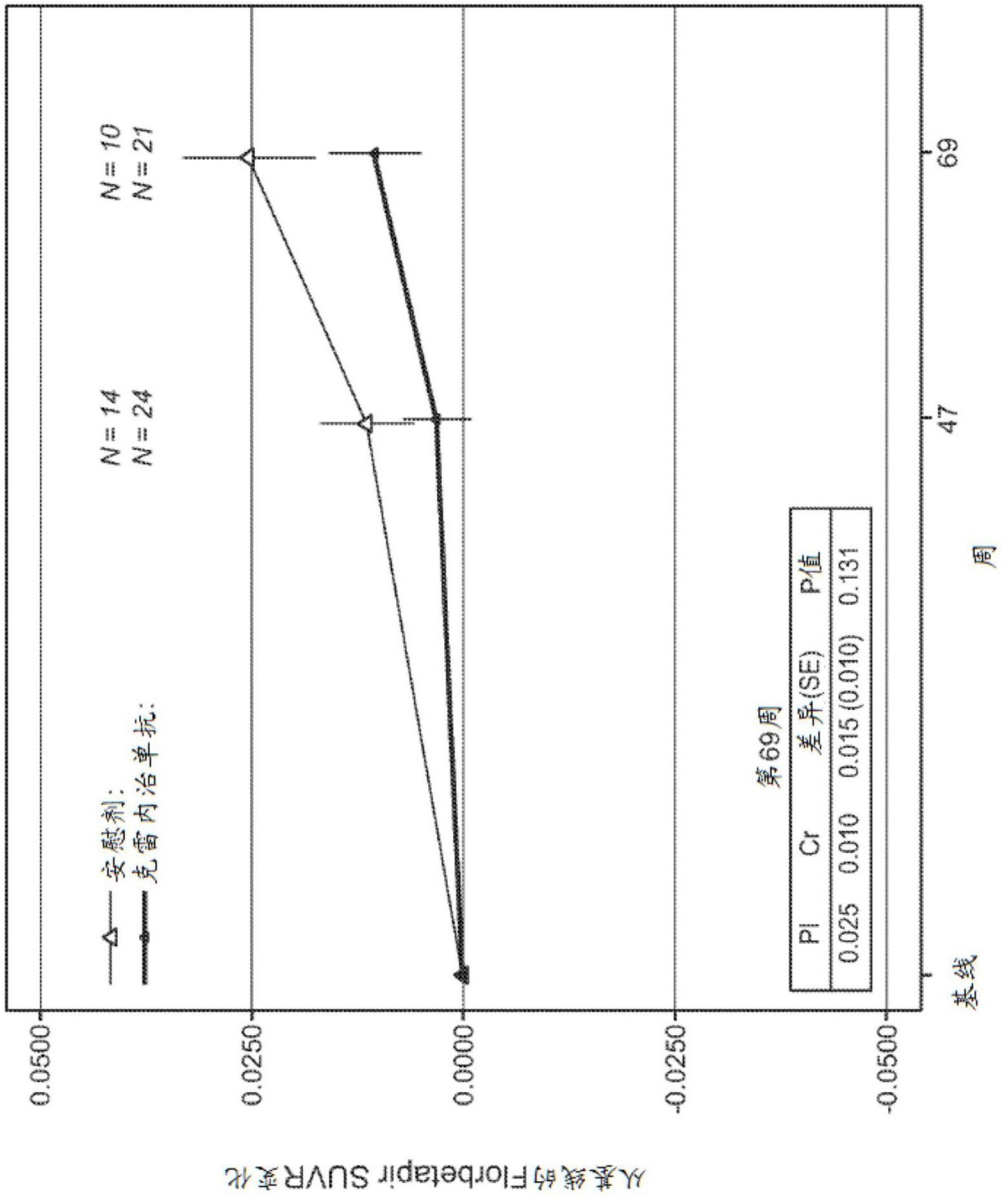

0.0500
0.0250
0.0000
-0.0250
-0.0500
基线
47
69
周
从基线的 Florbetapir SUVR 变化
安慰剂:
克雷内治单抗:
N = 14
N = 24
N = 10
N = 21
第69周
PI Cr 差异(SE) P值
0.025 0.010 0.015 (0.010) 0.131

图15A

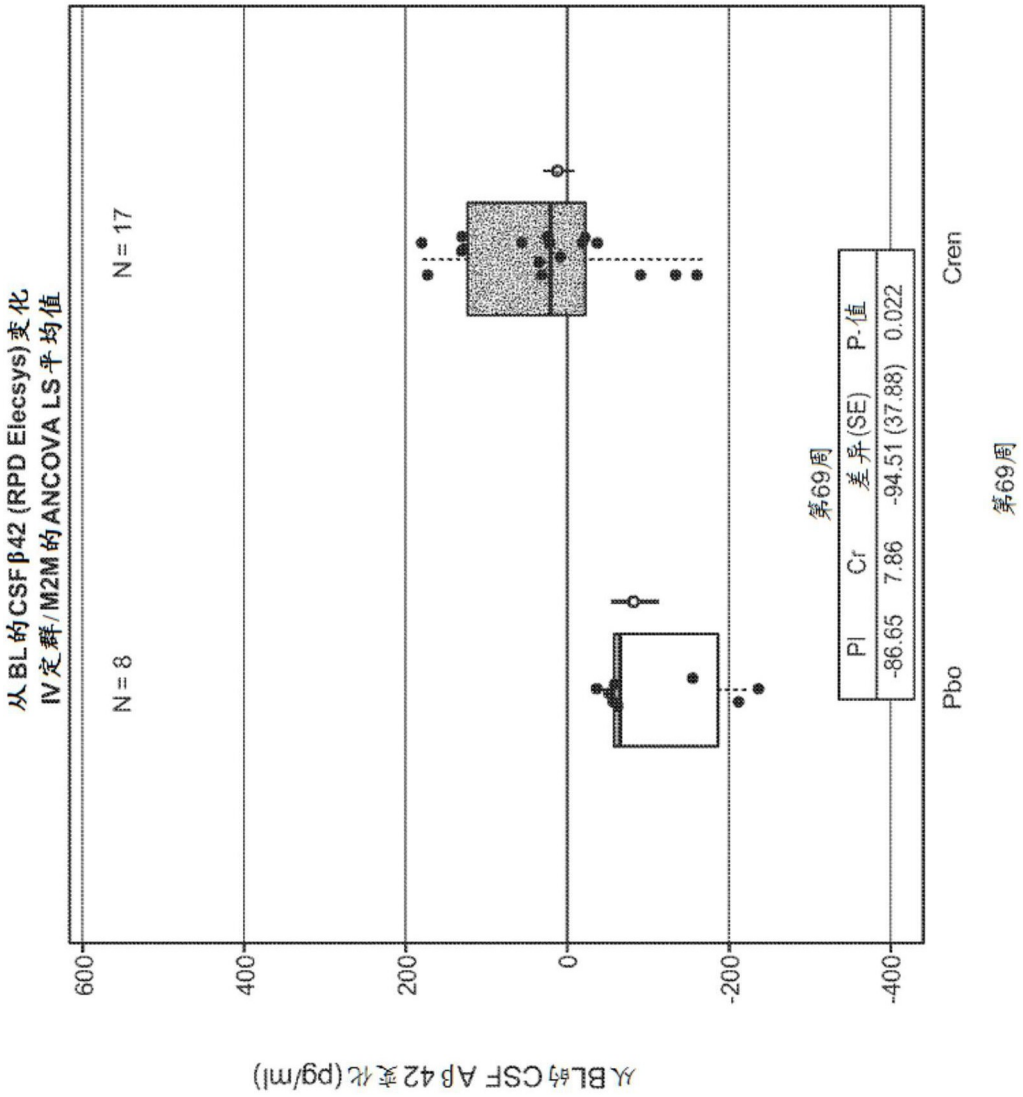

从 BL 的 CSF β42 (RPD Elecsys) 变化
IV 定群/M2M 的 ANCOVA LS 平均值
N = 8
N = 17
600
400
200
0
-200
-400
从 BL 的 CSF Aβ 42 变化 (pg/ml)
第69周
PI Cr 差异 (SE) P.值
-86.65 7.86 -94.51 (37.88) 0.022
Pbo
Cren
第69周

图15B