ANTIBODY FORMULATIONS AND METHODS

RELATED APPLICATIONS

Priority is claimed to U.S. Provisional Application No. 61/551,406, filed 25 October 2011, which is incorporated by reference herein in its entirety.

TECHNICAL FIELD

The invention resides in the technical fields of immunology and medicine.

BACKGROUND OF THE INVENTION

Amyloidosis is a general term that describes a number of diseases characterized by the existence of pathological forms of amyloid proteins, often involving extracellular deposition of protein fibrils, which form numerous "amyloid deposits" or "amyloid plaques," which may occur in local sites or systematically. These deposits or plaques are composed primarily of a naturally occurring soluble protein or peptide, assembled into extensive insoluble deposits 10-100 μm in diameter in a variety of tissue sites. The deposits are composed of generally lateral aggregates of fibrils that are approximately 10-15 nm in diameter. Amyloid fibrils produce a characteristic apple green birefringence in polarized light, when stained with Congo Red dye. Generally, the fibrillar composition of these deposits is an identifying characteristic for the various forms of amyloid disease.

The peptides or proteins forming the plaque deposits are often produced from a larger precursor protein. More specifically, the pathogenesis of amyloid aggregates such as fibril deposits generally involves proteolytic cleavage of an "abnormal" precursor protein into fragments that aggregate into anti-parallel β pleated sheets.

Systemic amyloidoses are a complex group of diseases caused by tissue deposition of misfolded proteins that result in progressive organ damage. The most common type, AL amyloidosis or primary amyloidosis, involves a hematological disorder caused by clonal plasma cells that produce misfolded immunoglobulin light chains. Overproduction of misfolded light chain by plasma cells results in deposits of abnormal AL protein (amyloid), in the tissues and organs of individuals with AL amyloidosis. Clinical features of AL amyloidosis include a

constellation of symptoms and organ dysfunction that can include cardiac, renal, and hepatic dysfunction, gastrointestinal involvement, neuropathies and macroglossia. The mechanisms by which amyloidogenic immunoglobulin light chains result in organ dysfunction are not well characterized, however, it is hypothesized that both amyloid deposits and prefibrillar aggregates may contribute to cytotoxic effects on organs observed in patients with AL amyloidosis. AL amyloidosis is a disease entity of its own, although AL amyloidosis can occur concurrently in a small subset (up to 15%) of patients with multiple myeloma.

AL amyloidosis is a rare disorder with an estimated incidence of 8 in 1,000,000 people. Only 1200 to 3200 new cases of AL amyloidosis are reported each year in the United States. Two thirds of patients with AL amyloidosis are male and less than 5% of patients are under 40 years of age. Both the causes and origins of AL amyloidosis remain poorly understood.

Current treatment of patients with AL amyloidosis is aimed at reducing or eliminating the bone marrow disorder, *i.e.* the plasma cells that are responsible for producing the light chains, thereby limiting or halting the production of amyloid. The most aggressive treatment options include stem cell transplant and high-dose chemotherapy for those patients who can tolerate it. Other treatment regimens include combinations of drugs often used to treat hematological malignancies, such as melphalan, prednisone, dexamethasone and proteosome inhibitors such as bortezomib, in an attempt to reduce light chain production. There are no currently approved treatments for AL amyloidosis that directly target potentially toxic forms of the amyloidogenic proteins.

A different form of systemic amyloidosis, AA amyloidosis or secondary amyloidosis, occurs "secondarily" as a result of other illness, such as chronic inflammatory diseases (for example, rheumatoid arthritis and ankylosing spondylitis) or chronic infections (for example, tuberculosis or osteomyelitis). In secondary amyloidosis, the depositing amyloid protein is amyloid A protein, derived from an acute-phase protein serum amyloid A. The treatment of secondary amyloidosis is directed at treating the underlying illness.

Thus, there is a need for therapies to treat AA amyloidosis and AL amyloidosis, which directly target the pathological amyloid fibrils. The present invention provides pharmaceutical formulations of 2A4 and 7D8 antibodies, and chimeric and humanized versions thereof, which

show high affinity binding to both AL and AA amyloids due to a shared immunogenic epitope of the pathological forms of these proteins.

SUMMARY OF THE INVENTION

The present invention provides antibody formulations useful for prophylaxis and treatment of amyloid disease. In one aspect of the invention, a pharmaceutical formulation comprises (a) a chimeric or humanized version of antibody 2A4 (ATCC Accession Number PTA-9662) or of antibody 7D8 (ATCC Accession Number PTA-9468), or fragment thereof, which specifically competes for binding to antigen with 2A4 or 7D8, and/or which is directed to an epitope comprising AEDS (SEQ ID NO: 18), wherein the antibody is present at a concentration within the range from about 1 mg/mL to about 100 mg/mL; (b) histidine buffer present at a concentration within the range from about 20 mM to about 30 mM; (c) trehalose present at a concentration within the range from about 210 mM to about 250 mM; and (d) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight; wherein the formulation is characterized by a pH within the range from about 6 to about 7. For example, representative formulations of the invention comprise an antibody having a light chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 4 and/or a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 5. More particularly, such a formulation can comprise an antibody having a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NO: 14-16, for example, an antibody having a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as SEQ ID NO: 15.

Additional representative formulations of the invention comprise (a) an antibody having a light chain variable region comprising three complementarity determining regions set forth as SEQ ID NOs: 6, 7, and 8, and a heavy chain variable region comprising three complementarity regions set forth as SEQ ID NOs: 9, 10, and 11; and (b) an antibody having a light chain variable region comprising three complementarity determining regions set forth as SEQ ID NOs: 12, 7, and 8, and a heavy chain variable region comprising three complementarity regions set forth as SEQ ID NOs: 9, 10, and 11.

In representative formulations of the invention, the antibody is present at a concentration within the range from about 5 mg/mL to about 15 mg/mL (*e.g.*, about 10 mg/mL), or present at a concentration within the range from about 25-75 mg/mL (*e.g.*, 50 mg/mL).

In other representative formulations of the invention, histidine buffer is present at a concentration of about 25 mM. The histidine buffer can comprise L-histidine and L-histidine HCl monohydrate. For example, L-histidine can be used at a concentration within the range from about 16 mM to about 22 mM and L-histidine HCl monohydrate can be used at a concentration within the range from about 4 mM to about 8 mM.

In other representative formulations of the invention, trehalose is present at a concentration of about 230 mM.

Prepared as described herein, representative formulations of the invention (a) are characterized by an osmolality of about 300 mOsm/kg; (b) comprise less than about 10% of the antibody present as an aggregate in the formulation; (c) further comprise a bulking agent; (d) are sterile; and/or (e) are stable upon freezing and thawing.

In one aspect of the invention, a formulation comprises (a) an antibody comprising a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16, and which is present at a concentration of about 10 mg/mL; (b) a histidine buffer present at a concentration of about 25 mM; (c) trehalose present at a concentration of about 230 mM; (d) polysorbate 20 present at a concentration of about 0.2 g/L; and (e) a pH of about 6.5.

In another aspect of the invention, a pharmaceutical formulation comprises (a) an antibody, which is antibody 2A4 (ATCC Accession Number PTA-9662), antibody 7D8 (ATCC Accession Number PTA-9468), or a chimeric or humanized version of antibody 2A4 or of antibody 7D8, or fragment thereof, which specifically competes for binding to antigen with 2A4 or 7D8, and/or which is directed to an epitope comprising AEDS (SEQ ID NO: 18), wherein the antibody is present at a concentration within the range from about 50 mg/mL to about 100 mg/mL; (b) a buffer; (c) a non-reducing sugar; and (d) a non-ionic surfactant. In particular examples, the antibody of the disclosed formulations comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as SEQ ID NOs: 15.

In another aspect of the invention, the antibody formulations are lyophilized. For example, a representative lyophilized formulation can comprise: (a) a humanized version of antibody 2A4 (ATCC Accession Number PTA-9662) or antibody 7D8 (ATCC Accession Number PTA-9468) or antigen binding fragment thereof; (b) histidine; (c) trehalose; and (d) polysorbate 20. Lyophilized formulations can have a pH of between about 6 to about 7 when reconstituted, such as pH 6.5 when reconstituted. Lyophilized formulations typically comprise about 100 mg to about 1000 mg of the antibody. Lyophilized formulations typically comprise polysorbate 20 at a concentration within the range from about 0.005% to about 0.05% by weight. Following reconstitution, the lyophilized formulations yield an aqueous solution, for example, an aqueous solution comprising: (a) an antibody comprising a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16, and which is present at a concentration of about 10 mg/mL; (b) a histidine buffer present at a concentration of about 25 mM; (c) trehalose present at a concentration of about 230 mM; (d) polysorbate 20 present at a concentration of about 0.2 g/L; and (e) a pH of about 6.5. A representative lyophilized formulation comprises about 100 mg of the antibody following reconstitution with sterile water.

Also provided are nucleic acids encoding antibodies used to prepare the disclosed formulations. For example, such nucleic acids include nucleic acids comprising nucleotide sequences encoding an antibody light chain of SEQ ID NO: 13 and nucleic acids comprising nucleotide sequences encoding an antibody heavy chain of any one of SEQ ID NOs: 14-16. For example, the nucleotide sequences set forth as SEQ ID NO: 19 and SEQ ID NO: 20 (which is identical to SEQ ID NO: 19 and further includes a sequence encoding a signal peptide) each encode the humanized 2A4 light chain of SEQ ID NO: 13. As another example, the nucleotide sequences set forth as SEQ ID NO: 22 and SEQ ID NO: 23 (which is identical to SEQ ID NO: 22 and further includes a sequence encoding a signal peptide) each encode the humanized 2A4 heavy chain of SEQ ID NO: 15.

For the production of antibodies, the disclosed nucleic acids may be included in a vector, either singly or in combination (*e.g.*, a combination of a nucleic acid encoding a humanized 2A4 light chain and a nucleic acid encoding a humanized 2A4 heavy chain). For example, a vector can comprise a nucleic acid comprising a nucleotide sequence encoding any one of SEQ ID

NOs: 13-16, 21, and 24; a nucleic acid comprising the nucleotide sequence of any one of SEQ ID NOs: 19-20 and 22-23, or combinations thereof. Representative vectors of the invention include (a) a vector comprising a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 or 21 and a humanized 2A heavy chain set forth as SEQ ID NO: 15 or 24; (b) a vector comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) a vector comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23.

Also provided are host cells (*e.g.*, CHO cells) having stably incorporated into their genomes one or more of the nucleic acids disclosed herein. For example, a host cell can comprise in its genome a stably integrated nucleic acid comprising a nucleotide sequence encoding any one of SEQ ID NOs: 13-16, 21, and 24; a stably integrated nucleic acid comprising the nucleotide sequence of any one of SEQ ID NOs: 19-20 and 22-23, or combinations thereof. Representative host cells of the invention include (a) host cells comprising a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 or 21 and a humanized 2A heavy chain set forth as SEQ ID NO: 15 or 24; (b) host cells comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) host cells comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23.

The present invention also provides methods of preparing pharmaceutical formulations. In one aspect of the invention, such a method comprises (a) culturing mammalian cells having stably incorporated into their genome nucleic acids encoding the light and heavy chains of a murine, chimeric or humanized 2A4 antibody or of a murine, chimeric or humanized 7D8 antibody so that the cells secrete the antibody into the cell culture media, and purifying the antibody from the cell culture media; (b) and preparing a formulation comprising (i) a chimeric or humanized version of antibody 2A4 (ATCC Accession Number PTA-9662) or of antibody 7D8 (ATCC Accession Number PTA-9468), or fragment thereof, that specifically competes for binding to antigen with 2A4 or 7D8, wherein the antibody is present at a concentration within the range from about 1 mg/mL to about 100 mg/mL; (ii) histidine buffer present at a concentration

within the range from about 20 mM to about 30 mM; (iii) trehalose present at a concentration within the range from about 210 mM to about 250 mM; and (iv) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight; wherein the formulation is characterized by a pH within the range from about 6 to about 7. For example, in one aspect of the invention, mammalian cells having stably incorporated into their genomes nucleic acids encoding the light and heavy chains of a humanized 2A4 antibody are cultured. Mammalian cells useful for this purpose include (a) host cells having stably incorporated into their genomes a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 or 21 and a humanized 2A heavy chain set forth as SEQ ID NO: 15 or 24; (b) host cells having stably incorporated into their genomes a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) host cells having stably incorporated into their genomes a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23. In some aspects of the invention, the disclosed methods of preparing a pharmaceutical formulation include the additional step of evaluating at least one property of antibody in the formulation, such as physical stability, chemical stability, and/or biological activity.

Still further provided are methods of therapeutically or prophylactically treating a human patient having or at risk of having amyloidosis characterized by the presence of amyloid protein fibrils, the method comprising administering to the patient an effective dosage of a formulation of the invention. Patients amenable to treatment have an amyloid disease such as amyloid A amyloidosis, which is characterized by the presence of amyloid A protein fibrils, or AL amyloidosis, which is characterized by the presence of amyloid light chain-type protein fibrils. Patients having AL amyloidosis may also suffer from an associated dyscrasis of the B lymphocyte lineage, for example a malignancy such as multiple myeloma.

The disclosed therapeutic and prophylactic treatment methods include combination therapies (*i.e.*, administration of the disclosed antibody formulations with one or more additional drug substances) to thereby elicit synergistic results. The two or more drug substances are administered simultaneously or sequentially in any order, *i.e.*, a formulation of the invention is administered prior to administration of a second drug substance, concurrently with a second drug substance, or subsequent to administration of a second drug substance. For example, a

formulation of the invention can be administered concurrently or consecutively in combination with melphalan. As another example, a formulation of the invention can be administered concurrently or consecutively in combination with one or more of bortezomib, melphalan, lenalidomide and carfilzomib.

In accordance with the disclosed therapeutic and prophylactic treatment methods, formulations of the invention can be administered in multiple dosages, for example, at a frequency in a range of about daily to about annually, such as at a frequency in a range of about every other week to about every three months, or such as once a month. In one aspect, an antibody formulation of the invention is administered intravenously at a dose in a range from about 10 mg to about 5000 mg drug substance. For example, a formulation can be administered at a dose in a range from about 30 mg to about 2500 mg humanized 2A4 drug substance at a frequency in a range of about every other week to about every other month. Representative dosages used in the disclosed methods include 30 mg, 100 mg, 300 mg, 1000 mg, 2000 mg, and 2500 mg of humanized 2A4 drug substance.

In one aspect of the invention, a method of therapeutically or prophylactically treating a human patient having or at risk for having light chain (AL) amyloidosis characterized by the presence of amyloid fibrils, deposits or prefibrillar aggregates, comprises administering to the patient an effective dosage of a pharmaceutical formulation comprising: (a) an antibody comprising a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16, and which is present at a concentration of about 10 mg/mL; (b) a histidine buffer present at a concentration of about 25 mM; (c) trehalose present at a concentration of about 230 mM; (d) polysorbate 20 present at a concentration of about 0.2 g/L; and (e) a pH of about 6.5. In such a method, the dosage is typically from about 0.5 mg/kg to about 30 mg/kg of the antibody (*e.g.*, about 0.5 mg/kg to about 8 mg/kg, or about 8 mg/kg to about 30 mg/kg) administered intravenously or subcutaneously at a frequency of from about weekly to about quarterly (*e.g.*, once every 28 days).

The present invention further provides a pharmaceutical product comprising: (a) a vial comprising about 100 mg antibody in powder form; (b) instructions for reconstitution of the antibody; and (c) instructions for preparing the reconstituted antibody for infusion, wherein (i)

the antibody comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16; and (ii) the reconstitution instructions require reconstitution with water for injection to an extractable volume of 10 mL.

BRIEF DESCRIPTION OF THE DRAWINGS

Figures 1A-1B show various humanized 2A4 antibody light chain and heavy chain sequences. Bold and underlining, consensus sequence for N-linked glycosylation.

Figure 2 shows murine 2A4 and 7D8 light chain variable region (VL) and heavy chain variable region (VH) sequences. Double underlining, leader sequence; underlining, complementarity determining region (CDR) sequences.

Figure 3 shows humanized 2A4 version 3 light chain variable region (VL) and heavy chain variable region (VH) sequences. Lower case, back mutations.

Figures 4A-4B show nucleic acid sequences encoding humanized 2A4 version 3 heavy chain (FIG. 4A) and heavy chain (FIG. 4B) sequences. Single underline, leader sequence; no underline, variable region; double underline, constant region.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides antibody formulations useful for prophylaxis and treatment of amyloid disease. In one aspect of the invention, a pharmaceutical formulation comprises (a) a chimeric or humanized version of antibody 2A4 (ATCC Accession Number PTA-9662) or of antibody 7D8 (ATCC Accession Number PTA-9468), or fragment thereof, which specifically competes for binding to antigen with 2A4 or 7D8, and/or which is directed to an epitope comprising AEDS (SEQ ID NO: 18), wherein the antibody is present at a concentration within the range from about 1 mg/mL to about 100 mg/mL; (b) histidine buffer present at a concentration within the range from about 20 mM to about 30 mM; (c) trehalose present at a concentration within the range from about 210 mM to about 250 mM; and (d) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight; wherein the formulation is characterized by a pH within the range from about 6 to about 7.

In one aspect of the invention described herein, humanized 2A4 is an IgG1, kappa isotype version of murine 2A4. In the course of specificity characterization of humanized 2A4, the antibody was found to also react with high affinity and in a conformation-dependent manner with light chain in light chain amyloid fibrils, but not with free light chain in circulation.

The present invention provides methods for intravenous infusion of humanized 2A4 and/or humanized 7D8 antibodies to target misfolded amyloid protein in patients with AA amyloidosis and AL amyloidosis. Some humanized 2A4 antibodies specifically bind to pathologic amyloid forms of AL and SAA but do not bind to the parent molecules from which these pathologic forms are derived (SAA, native immunoglobulin light chain [LC], intact immunoglobulin [Ig]).

I. Pharmaceutical Formulations and Products

I.A. Characteristics

Provided herein are pharmaceutical formulations comprising a chimeric or humanized version of antibody 2A4 (ATCC Accession Number PTA-9662) or of antibody 7D8 (ATCC Accession Number PTA-9468), or fragment thereof, that specifically competes for binding to antigen (*i.e.*, human AA or AL protein) with 2A4 or 7D8, respectively, and/or that is directed to the epitope AEDS (SEQ ID NO: 18). Also provided are pharmaceutical formulations comprising murine antibody 2A4 or murine antibody 7D8, or fragments thereof. The antibody is present at a concentration within the range from about 1 mg/mL to about 100 mg/mL. The formulation is characterized by a pH within the range from about 6 to about 7 and comprises a histidine buffer at a concentration within the range from about 20 mM to about 30 mM, trehalose at a concentration within the range from about 210 mM to about 250 mM; and polysorbate 20 at a concentration within the range from about 0.005% to about 0.05% by weight.

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

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

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

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

In some formulations, the antibody comprises a light chain variable region comprising an amino acid sequence set forth as any one of SEQ ID NOs: 1, 2, or 4. In some formulations, the

antibody comprises a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 3 or 5. In some formulations, the antibody comprises a light chain variable region comprising an amino acid sequence set forth as any one of SEQ ID NOs: 1, 2, or 4 and a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 3 or 5. In some formulations, the antibody comprises a light chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 1 and a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 3. In some formulations, the antibody comprises a light chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 4 and a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 5. In some formulations, the antibody comprises a light chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 2 and a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 3.

In some formulations, the antibody comprises a light chain variable region comprising three complementarity determining regions set forth as SEQ ID NOs: 6, 7, and 8, and a heavy chain variable region comprising three complementarity regions set forth as SEQ ID NOs: 9, 10, and 11. In other formulations, the antibody comprises a light chain variable region comprising three complementarity determining regions set forth as SEQ ID NOs: 12, 7, and 8, and a heavy chain variable region comprising three complementarity regions set forth as SEQ ID NOs: 9, 10, and 11.

In other formulations of the present invention, the antibody comprises light chain and heavy chain variable regions of a murine, chimeric, or humanized 2A4 antibody, or of a murine, chimeric, or humanized 7D8 antibody, as described in U.S. Patent No. 7,928,203 and PCT International Publication No. WO 2009/086539, each of which is incorporated herein by reference in its entirety, and the light chain and heavy chain variable region sequences described in the referenced patent and publication are specifically incorporated by reference herein.

In some formulations, the antibody comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 or 21 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16 and 24. The antibody can include, or not include, the leader sequences of the above-noted light chain and heavy chain amino acid sequences.

In other formulations, the antibody is a fragment of a 2A4 or 7D8 antibody, including chimeric and humanized versions thereof, such as a Fab fragment, a Fab' fragment, a $F(ab')_2$ fragment, a Fv fragment or a ScFv fragment.

In some aspects of the invention, the antibody specifically binds to aggregated amyloid protein without specifically binding to monomeric amyloid protein (*e.g.*, at least a 10-fold and usually at least 100-fold lower specific binding affinity for monomeric forms of the amyloid protein).

In some formulations, the antibody is present at a concentration within the range from about 5 mg/mL to about 100 mg/mL. In some formulations, the antibody is present at a concentration within the range from about 5 mg/mL to about 15 mg/mL. In some formulations, the antibody is present at a concentration within the range from about 25 mg/mL to about 75 mg/mL. For example, the antibody may be present at a concentration of about 10mg/mL, or present at a concentration of about 50 mg/mL. The antibody may be present in a sterile liquid dosage form of about 50 mg/vial to about 500 mg/vial, or greater. For example, the antibody may be present in a sterile liquid dosage form of about 100 mg/vial.

Antibodies used in the disclosed formulations can be coupled with a therapeutic moiety, such as a cytotoxic agent, a radiotherapeutic agent, an immunomodulator, a second antibody (*e.g.*, to form an antibody heteroconjugate), or any other biologically active agent that facilitates or enhances the activity of a chimeric or humanized 2A4 or a chimeric or humanized 7D8 antibody. Representative therapeutic moieties include agent known to be useful for treatment, management, or amelioration of amyloid disease or symptoms of amyloid disease.

Antibodies used in the disclosed formulations can also be coupled with a detectable label, for example, as useful for diagnosing an amyloid disorder, for monitoring progression of amyloid disease, and/or for assessing efficacy of treatment. Antibodies formulated as described are particularly useful for performing such determinations in subjects having or being susceptible to AA amyloidosis or AL amyloidosis, or in appropriate biological samples obtained from such subjects. Representative detectable labels that may be coupled or linked to a humanized 2A4 antibody or humanized 7D8 antibody include various enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such streptavidinlbiotin and avidin/biotin; fluorescent materials, such as but umbelliferone,

fluorescein, fluorescein isothiocynate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as luminol; bioluminescent materials, such as luciferase, luciferin, and aequorin; radioactive materials, such as but not limited to iodine (^{131}I, ^{125}I, ^{123}I, ^{121}I,), carbon (^{14}C), sulfur (^{5}S), tritium (^{3}H), indium (^{115}In, ^{113}In, ^{112}In, ^{111}In,), and technetium (^{99}Tc), thallium (^{201}Ti), gallium (^{68}Ga, ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm, ^{177}Lu, ^{159}Gd, ^{149}Pm, ^{140}La, ^{175}Yb, ^{166}Ho, ^{90}Y, ^{47}Sc, ^{186}Re, ^{188}Re, ^{142}Pr, ^{105}Rh, ^{97}Ru, ^{68}Ge, ^{57}Co, ^{65}Zn, ^{85}Sr, ^{32}P, ^{153}Gd, ^{169}Yb, ^{51}Cr, ^{54}Mn, ^{75}Se, ^{113}Sn, and ^{117}Tin; positron emitting metals using various positron emission tomographies, nonradioactive paramagnetic metal ions, and molecules that are radiolabelled or conjugated to specific radioisotopes.

Therapeutic moieties and/or detectable substances may be coupled or conjugated directly to a murine, chimeric or humanized 2A4 antibody or a murine, chimeric or humanized 7D8 antibody, or indirectly, through an intermediate (*e.g.*, a linker) using techniques known in the art. *See e.g.*, Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies 84: Biological And Clinical Applications, Pinchera et al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., *Immunol. Rev.*, 1982, 62:119-58.

Antibodies used in the disclosed formulations also include modified forms of murine, chimeric or humanized 2A4 antibodies, or murine, chimeric or humanized 7D8 antibodies, which have increased *in vivo* half-lives relative to the corresponding unmodified antibodies. Such modified forms may be prepared, for example, by glycosylation, acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, *etc.* As one example, representative methods for antibody half-life extension are described in PCT International Publication No. WO 02/060919.

The present invention encompasses antibody formulations having stability at 38°C-42°C as assessed by high performance size exclusion chromatography (HPSEC) for at least about 30 days, formulations having stability at 20°C-24°C for at least about 1 year, and formulations having stability at 2°C-4°C for at least about 3 years. More particularly, the disclosed formulations exhibit low to undetectable levels of antibody aggregation and/or fragmentation, or a low or undetectable increase of antibody fragmentation and/or aggregation above an initial level (*e.g.*, less than about 10% aggregation. A formulation having low to undetectable levels of fragmentation contains at least about 80%, 85%, 90%, 95%, 98%, or 99%, of the total protein, for example, in a single peak as determined by high performance size exclusion chromatography (HPSEC), or in two (2) peaks (one corresponding to each of the antibody heavy chains and antibody light chains) by reduced Capillary Gel Electrophoresis (rCGE), representing the non-degraded antibody, and containing no other single peaks having more than 5%, more than 4%, more than 3%, more than 2%, more than 1%, or more than 0.5% of the total protein each. A formulation having low to undetectable levels of aggregation contains no more than about 15%, no more than about 10%, no more that about 5%, no more than about 4%, no more than about 3%, no more than about 2%, no more than about 1%, or no more than about 0.5% aggregation by weight protein as measured by high performance size exclusion chromatography (HPSEC). For example, in some formulations, less than about 10% of the anti-amyloid antibody is present as an aggregate. Stable formulations of the invention also show little or no loss of biological activity(ies) of a chimeric or humanized 2A4 or chimeric or humanized 7D8, for example binding affinity measurable by ELISAs and/or additional functional assays, such as at least about at least about 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% of an initial measurable value of a given activity.

The histidine buffer may be present in some formulations at a concentration of about 25 mM. In some formulations, the histidine buffer comprises L-histidine and L-histidine HCl monohydrate. For example, in some formulations, L-histidine is present at a concentration within the range from about 16 mM to about 22 mM and L-histidine HCl monohydrate is present at a concentration within the range from about 4 mM to about 8 mM.

In some formulations, trehalose is present at a concentration from about 210 mM to about 250 mM, for example, about 230 mM. In some formulations, a different non-reducing sugar is used, such as sucrose, mannitol, or sorbitol.

In some formulations, polysorbate 20 is present at a concentration within the range of about from about 0.005% to about 0.05% by weight, for example, 0.005%, 0.01%, 0.015%, 0.02%, 0.025%, 0.03%, 0.035%, 0.04%, 0.045%, or 0.05%. Alternatively, in some formulations, polysorbate 20 is present at a concentration within the range of about from about 0.05 g/L, 0.1 g/L, 0.15 g/L, 0.2 g/L, 0.25 g/L, 0.3 g/L, 0.35 g/L, 0.4 g/L, 0.45 g/L, or 0.5 g/L. Some formulations include polysorbate 20 at a concentration of 0.2 g/L.

Some formulations are characterized by a pH within the range of about 6-7, for example, a pH of 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, or 7.0. Some formulations have a pH of about 6.5.

Some formulations are characterized by an osmolality of about 300 mOsm/kg.

A bulking agent may also be included some formulations.

Typically, the formulations are sterile, for example, as accomplished by sterile filtration using a 0.2 μm or a 0.22 μm filter. The formulations of the invention are also generally stable upon freezing and thawing.

Optionally, formulations of the invention may further comprise other excipients, such as saccharides, polyols, and amino acids (*e.g.*, arginine, lysine, and methionine). In other aspects, the present invention also provides formulations substantially free of surfactant, inorganic salts, additional sugars, and/or other excipients, *i.e.*, less than about less than 0.0005%, less than 0.0003%, or less than 0.0001% of such compounds.

An exemplary formulation comprises an antibody comprising a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14, 15, or 16, which is present at a concentration of about 10 mg/mL, a histidine buffer present at a concentration of about 25 mM, trehalose present at a concentration of about 230 mM; polysorbate 20 present at a concentration of about 0.2 g/L, and a pH of about 6.5.

I.B. Preparation of Pharmaceutical Formulations

The present invention also provides methods of preparing pharmaceutical formulations. In one aspect of the invention, such a method comprises (a) culturing mammalian cells having stably incorporated into their genome nucleic acids encoding the light and heavy chains of murine antibody 2A4 (ATCC Accession Number PTA-9662) or of antibody 7D8 (ATCC Accession Number PTA-9468), or of chimeric or humanized versions thereof, so that the cells secrete the antibody into the cell culture media, and purifying the antibody from the cell culture media; (b) and preparing a formulation comprising (i) the purified antibody present at a concentration within the range from about 1 mg/mL to about 100 mg/mL; (ii) histidine buffer present at a concentration within the range from about 20 mM to about 30 mM; (iii) trehalose present at a concentration within the range from about 210 mM to about 250 mM; and (iv) polysorbate 20 present at a concentration within the range from about 0.005% to about 0.05% by weight; wherein the formulation is characterized by a pH within the range from about 6 to about 7.

In some aspects of the invention, the disclosed methods of preparing a pharmaceutical formulation include the additional step of evaluating at least one property of antibody in the formulation selected from the group consisting of the physical stability, chemical stability and biological activity.

For example, in one aspect of the invention, mammalian cells are cultured for the production of antibodies, wherein the mammalian cells have stably incorporated into their genomes nucleic acids encoding the light and heavy chains of a humanized 2A4 antibody. Mammalian cells useful for this purpose include (a) host cells having stably incorporated into their genomes a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 or 21 and a humanized 2A heavy chain set forth as SEQ ID NO: 15 or 24; (b) host cells having stably incorporated into their genomes a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) host cells having stably incorporated into their genomes a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23.

For the production of antibodies, the disclosed nucleic acids are included in a vector. In some examples, the vector contains the nucleic acid encoding murine 2A4 of 7D8 antibody, or a chimeric or humanized version thereof, operably linked to a suitable control sequence capable of effecting the expression of the DNA in a host cell. Such control sequences include a promoter to effect transcription (*e.g.*, a constitutive promoter or inducible promoter as known in the art), an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, enhancers, polyadenylation signals, and sequences to control the termination of transcription and translation. The vector may be a plasmid, a phage particle (*e.g.*, a viral vector such as adenovirus, adeno-associated-virus, retrovirus, herpes virus, vaccinia virus, lentivirus, poxvirus and cytomegalovirus vectors), or simply a genomic insert. Once transformed into a suitable host, the antibody nucleic acids may integrate into the genome of the host, or the vector may replicate and function independently of the host genome.

The disclosed nucleic acids are included in a vector either singly or in combination (*e.g.*, a combination of a nucleic acid encoding an antibody light chain and a nucleic acid encoding an antibody heavy chain). For example, a vector can comprise a nucleic acid comprising a nucleotide sequence encoding any one of SEQ ID NOs: 13-16, 21, or 24; a nucleic acid comprising the nucleotide sequence of any one of SEQ ID NOs: 19-20 and 22-23, or combinations thereof. Representative vectors of the invention include (a) a vector comprising a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 and a humanized 2A heavy chain set forth as SEQ ID NO: 15; (b) a vector comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) a vector comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23.

Host cells useful for preparing antibody formulations of the invention include mammalian cells, including cells of human origin, such as monkey kidney cells, human embryonic kidney cells, baby hamster kidney (BHK) cells, Chinese hamster ovary cells (CHO) cells, mouse sertoli cells, human cervical carcinoma (HeLa) cells, canine kidney cells, human lung cells, human liver cells, mouse mammary tumor cells, and NS0 cells. For example, a host cell can comprise in its genome a stably integrated nucleic acid comprising a nucleotide

sequence encoding any one of SEQ ID NOs: 13-16, 21, and 24; a stably integrated nucleic acid comprising the nucleotide sequence of any one of SEQ ID NOs: 19-20 and 22-23, or combinations thereof. Representative host cells of the invention include (a) host cells comprising a nucleic acid sequence encoding a humanized 2A4 light chain set forth as SEQ ID NO: 13 or 21 and a humanized 2A heavy chain set forth as SEQ ID NO: 15 or 24; (b) host cells comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 19 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 22; and (c) host cells comprising a nucleic acid having the nucleotide sequence of SEQ ID NO: 20 and a nucleic acid having the nucleotide sequence of SEQ ID NO: 23.

In another aspect of the invention, a chimeric or humanized 2A4 antibody or a chimeric or humanized 7D8 antibody is prepared by chemical synthesis and then used in the disclosed formulations.

Antibodies used to prepare the disclosed formulations are typically isolated or purified, *i.e.*, substantially free of cellular material or other contaminating proteins from the cells in which they are produced, or substantially free of chemical precursors or other chemicals when chemically synthesized. For example, an antibody that is substantially free of cellular material includes preparations of the antibody having less than about 30%, 25%, 20%, 15%, 10%, 8%, 5%, 2%, 1%, 0.5%, 0.1%, or less (by dry weight) of contaminating protein. When an antibody is recombinantly produced, it is also substantially free of culture medium such that culture medium represents less than about 30%, 25%, 20%, 15%, 10%, 8%, 5%, 2%, 1%, 0.5%, 0.1%, or less, of the volume of the protein preparation. When an antibody is produced by chemical synthesis, it is preferably substantially free of or separated from chemical precursors or other chemicals involved in the synthesis of the protein. Accordingly, such antibody preparations have less than about 30%, 25%, 20%, 15%, 10%, 8%, 5%, 2%, 1%, 0.5%, 0.1%, or less (by dry weight) of chemical precursors or compounds other than the antibody drug substance. Purification of recombinantly expressed antibody can utilize any of a number of methods known in the art, such as, for example, affinity chromatography, acid treatment, depth filtration, anion exchange chromatography, cation exchange chromatography, nanofiltration, ultrafiltration, dialysis and diafiltration.

The purified antibody drug substance can be adjusted to a solution comprising any of the

formulations described herein, diluted to the desired concentration and stored until ready for use. Optionally, the formulation can be stored in concentrated form until ready for use. Liquid formulations can be stored in frozen form, under refrigeration or at room temperature, depending upon their stability profile, which can be determined empirically. In some instances a further filtration step is applied. Some of the formulations described herein may be lyophilized and stored in powder form. Lyophilized formulations can be stored in frozen form, under refrigeration or at room temperature, depending upon their stability profile, which can be determined empirically. For example, the lyophilized formulations can be stored at a temperature of about 2°C to 8°C. In such cases, the formulation would be reconstituted prior to administration to a patient to yield a liquid formulation having the antibody and excipients present in the concentrations described herein. In some cases, the formulation is reconstituted in sterile water. In some cases, the formulation is reconstituted and added to an infusion bag for administration to the patient. The reconstituted formulation can be stored under refrigeration or at room temperature prior to administration to a patient for a time consistent with the stability profile. Lyophilization and reconstitution techniques are known in the art and described in the Examples.

Thus, the present invention also encompasses pharmaceutical products comprising lyophilized antibody drug substance and instructions for reconstitution and use. For example, a representative pharmaceutical product can comprise: (a) a vial comprising about 100 mg antibody in powder form; (b) instructions for reconstitution of the antibody; and (c) instructions for preparing the reconstituted antibody for infusion, wherein (i) the antibody comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16; and (ii) the reconstitution instructions require reconstitution with water for injection to an extractable volume of 10 mL.

II. Methods of Diagnosis and Treatment

Also provided are methods of therapeutically or prophylactically treating a human patient having or at risk of having amyloidosis characterized by the presence of amyloid protein fibrils,

the method comprising administering to the patient an effective dosage of any of the formulations described herein.

II.A. Subjects Amenable to Diagnosis and Treatment

Humanized 2A4 drug substance is to be used for the treatment of systemic amyloidosis, such as amyloidoses involving either amyloid light chain AL or amyloid A (AA) proteins. Systemic amyloidoses are a complex group of diseases caused by tissue deposition of misfolded proteins that result in progressive organ damage. The most common type, AL amyloidosis or primary amyloidosis, involves a hematological disorder caused by clonal plasma cells that produce misfolded immunoglobulin light chains. Overproduction of misfolded light chain by plasma cells results in deposits of abnormal AL protein (amyloid), in the tissues and organs of individuals with AL amyloidosis. Clinical features of AL amyloidosis include a constellation of symptoms and organ dysfunction that can include cardiac, renal, and hepatic dysfunction, GI involvement, neuropathy's and macroglossia. A different form of systemic amyloidosis, AA amyloidosis or secondary amyloidosis, occurs "secondarily" as a result of other illness, such as chronic inflammatory diseases (for example, rheumatoid arthritis and ankylosing spondylitis) or chronic infections (for example, tuberculosis or osteomyelitis). In secondary amyloidosis, the depositing amyloid protein is amyloid A protein, derived from an acute-phase protein serum amyloid A.

Peripheral amyloidosis is be amenable to this type of amyloid-specific immunotherapy through antibody targeting of a neo-epitope that has been identified in AA amyloid, as well as in AL amyloid. Studies in animal models of both AA and AL have demonstrated that significant positive therapeutic effects may be possible at reasonable doses of antibody.

Subjects or patients amenable to treatment using the disclosed antibody formulations include individuals at risk of disease but not showing symptoms, as well as patients presently showing symptoms of amyloid disease. Therefore, the present methods can be administered prophylactically to the general population without the need for any assessment of the risk of the subject patient. For example, the present methods are especially useful for individuals who do have a known genetic risk autoimmune disorders. Such individuals include those having relatives who have experienced this disease and those whose risk is determined by analysis of

genetic or biochemical markers. As another example, patients suffering from AA amyloidosis can be asymptomatic for a prolonged period of time, such that clinical diagnosis of AA amyloidosis is often delayed or missed until the amyloid deposits are extensive. For those patients who are symptomatic, it is estimated that only 53% of the cases are diagnosed. *See e.g.*, L.E.K. Consulting, Independent Market Research (2003). Prophylactic administration disclosed antibody formulations may reduce the incidence of AA amyloidosis.

The present methods are especially useful for individuals who do have a known risk of, are suspected to have, or have been diagnosed with AA amyloidosis or AL amyloidosis. Such individuals include but are not limited to those having chronic inflammatory diseases, inherited inflammatory diseases, and chronic microbial infections, such as rheumatoid arthritis, juvenile chronic arthritis, ankylosing spondylitis, psoriasis, psoriatic arthropathy, Reiter's syndrome, Adult Still's disease, Behcet's syndrome, Crohn's disease, Familial Mediterranean Fever, leprosy, tuberculosis, bronchiectasis, decubitus ulcers, chronic pyelonephritis, osteomyelitis, Whipple's disease, myeloma, macroglobulinemia, immunocyte dyscrasia, monoclonal gammopathy, occult dyscrasia. Chronic inflammatory and infectious conditions are prerequisite to the development of AA amyloidosis and AL amyloidosis manifested by local nodular amyloidosis can be associated with chronic inflammatory diseases. Individuals who do have known risk of AA amyloidosis also include but are not limited to those having malignant neoplasms as Hodgkin's lymphoma, renal carcinoma, carcinomas of gut, lung and urogenital tract, basal cell carcinoma, and hairy cell leukemia. Additionally, individuals with known risk of AA amyloidosis also include but are not limited to those having lymphoproliferative disorders such as Castleman's Disease. Some of such patients have AA amyloidosis characterized by the presence of amyloid A protein fibrils. Some of such patients have AL amyloidosis characterized by the presence of amyloid light chain-type protein fibrils. Some patients have systemic organ dysfunction attributed to AL amyloidosis, including dysfunction of the heart, kidney, liver, peripheral nervous system, gastrointestinal system, autonomic nervous system, lung, and/or soft tissue or lymphatic system.

Patients amenable to treatment also include those patients who have received, are currently receiving, or will later receive an alternate therapy, for treatment of amyloid disease or an associated condition, such as, inflammatory diseases, chronic microbial infections, malignant

neoplasms, inherited inflammatory diseases, and lymphoproliferative disorders. For example, patients may also receive or have received one or more of the therapeutic agents identified herein with respect to combination therapies. As a particular example, patients suffering from AL may also receive or have received bortezomib, melphalan, lenalidomide and/or carfilzomib. For those patients who have previously received alternate therapies for the treatment of amyloid disease, such therapies may or may not have been successful by the relevant clinical measures.

II.B. Treatment Regimes

As used herein, the terms "treat" and "treatment" refer to the alleviation or amelioration of one or more symptoms or effects associated with the disease, prevention, inhibition or delay of the onset of one or more symptoms or effects of the disease, lessening of the severity or frequency of one or more symptoms or effects of the disease, and/or increasing or trending toward desired outcomes as described herein.

Desired outcomes of the treatments disclosed herein vary according to the amyloid disease and patient profile and are readily determinable to those skilled in the art. Generally, desired outcomes include measurable indices such as reduction or clearance of pathologic amyloid fibrils, decreased or inhibited amyloid aggregation and/or deposition of amyloid fibrils, and increased immune response to pathologic and/or aggregated amyloid fibrils. Desired outcomes also include amelioration of amyloid disease-specific symptoms. For example, desired outcomes for the treatment of AL amyloidosis include a decrease in the incidence or severity of known symptoms, including organ dysfunction, peripheral and autonomic neuropathy, carpal tunnel syndrome, macroglossia, restrictive cardiomyopathy, arthropathy of large joints, immune dyscrasias, myelomas, as well as occult dyscrasias. As another example, desired outcomes for the treatment of AA include a decrease in associated inflammation, arthritis, psoriasis, microbial infection, malignancy, or symptoms of other preexisting or coexisting disease to which the AA amyloidosis is secondary.

Desired outcomes of the disclosed therapies are generally quantifiable measures as compared to a control or baseline measurement. As used herein, relative terms such as "improve," "increase," or "reduce" indicate values relative to a control, such as a measurement in the same individual prior to initiation of treatment described herein, or a measurement in a

control individual or group. A control individual is an individual afflicted with the same amyloid disease as the individual being treated, who is about the same age as the individual being treated (to ensure that the stages of the disease in the treated individual and the control individual are comparable), but who has not received treatment using the disclosed antibody formulations. In this case, efficacy of the disclosed antibody formulations is assessed by a shift or trend away from measurable indices in the untreated control. Alternatively, a control individual is a healthy individual, who is about the same age as the individual being treated. In this case, efficacy of the disclosed antibody formulations is assessed by a shift or trend toward from measurable indices in the healthy control. Changes or improvements in response to therapy are generally statistically significant and described by a p-value less than or equal to 0.1, less than 0.05, less than 0.01, less than 0.005, or less than 0.001 may be regarded as significant.

In both asymptomatic and symptomatic patients, treatment according to the disclosed methods can begin at any time before or after the diagnosis of the underlying AA or AL amyloid diseases. Treatment typically entails multiple dosages over a period of time. Treatment can be monitored by assaying antibody, or employing radiolabeled SAP Scintigraphy over time. If the response falls, a booster dosage may be indicated. The response of patients with AL amyloidosis to treatment can be monitored by assessing cardiac markers, such as NT-proBNP and/or troponin, serum creatine, and/or alkaline phosphatase; by performing serum free light chain (SFLC) assays, quantitative immunoglobulin assays, biopsies, serum protein electrophoresis (SPEP), urine protein electrophoresis (UPEP), serum, urine immunofixation electrophoresis (IFE), and/or organ imaging techniques. An exemplary complete response (CR) can be determined from response criteria including negative IFE of serum and urine, normal κ/λ ration and/or <5 % plasma cells in bone marrow. An exemplary very good partial response (VGPR) can be determined from a dFLC of < 40 mg/L. An exemplary partial response (PR) can be determined from a dFLC decrease of $\geq$ 50%. In the kidney, a response to treatment can be determined, for example, from a $\geq$ 50% reduction (*e.g.*, > 0.5g/24 hours) in 24 hour urine protein excretion in the absence of either a reduction in eGFR of $\geq$ 25% or an increase in serum creatine of $\geq$ 0.5 mg/dL. In the liver, a response to treatment can be determined, for example, from a $\geq$ 50% reduction in initially elevated alkaline phosphatase or a $\geq$ 2 cm reduction in liver size on CT scan or MRI. In the heart, a response to treatment can be determined, for example, from a $\geq$ 30%

and 300 ng/L reduction in NT-proBNP in patients with baseline of NT-proBNP of > 650 ng/L.

The antibody formulation can be administered intravenously in dosage ranges from about 10 mg to about 5000 mg for the patient in question, such as, for example, about 10 mg, about 30 mg, about 100 mg, about 300 mg, about 1000 mg, about 2000 mg, or about 2500 mg. The antibody formulation can also be administered intravenously in dosage ranges from about 0.1 mg/kg to about 50 mg/kg, or from about 0.5 mg/kg to about 30 mg/kg, of the host body weight. For example, dosages can be about 0.5 mg/kg body weight, about 1.0 mg/kg, about 1.5 mg/kg, about 2.0 mg/kg, about 4.0 mg/kg, about 5.0 mg/kg, about 8.0 mg/kg, about 10 mg/kg, about 15 mg/kg, about 16 mg/kg, about 20 mg/kg, about 25 mg/kg, or about 30 mg/kg body weight. Dose escalation for an individual patient can occur at the discretion of the prescriber in the absence of any clinically significant occurrence that the prescriber might reasonably believe would present an undue safety risk for the patient, such as, for example, Grade $\geq$ 3 non-hematologic toxicity, Grade $\geq$ 3 nausea, vomiting or diarrhea uncontrolled by maximum antiemetic/anti-diarrhea therapy, Grade 4 neutropenia lasting > 7 days in the absence of growth factor support, Grade 3 or 4 neutropenia of any duration accompanied with fever $\geq$ 38.5°C and/or systemic infection, or other Grade $\geq$ 4 hematologic toxicity.

Antibody is usually administered on multiple occasions. An exemplary treatment regime entails administration once per every two weeks, once a month, or once every 3 to 6 months. For example, patients can receive the antibody formulation once every four weeks as a cycle, for example every twenty-eight days. The dosing frequency can be adjusted depending on the pharmacokinetic profile of the antibody formulation in the patient. For example, the half-life of the antibody may warrant a two week frequency of dosing. In some methods, two or more monoclonal antibodies with different binding specificities are administered simultaneously, in which case the dosage of each antibody administered falls within the ranges indicated. Intervals between single dosages can be weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of antibody to amyloid protein (*e.g.*, AA) in the patient. In some methods, dosage is adjusted to achieve a plasma antibody concentration of about 1–1000 μg/ml or about 25–300 μg/ml. Alternatively, antibody can be administered as a sustained release formulation, in which case less frequent administration is required.

Dosage and frequency vary depending on the half-life of the antibody in the patient. In

general, human antibodies show the longest half life, followed by humanized antibodies, chimeric antibodies, and nonhuman antibodies. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. Some patients continue to receive treatment for the rest of their lives. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, until a partial or complete response is achieved, and/or until the patient shows lessening or amelioration of symptoms of disease. Thereafter, the patent can be administered a prophylactic regime.

The formulations disclosed herein may be provided in a dosage form that is suitable for parenteral (*e.g.*, intravenous, intramuscular, subcutaneous) administration. As appropriate for particular applications, the formulation may be alternately provided in a dosage suitable for rectal, transdermal, nasal, vaginal, inhalant, ocular or other administration. The pharmaceutical formulations are typically prepared according to conventional pharmaceutical practice. *See e.g.*, Remington: The Science and Practice of Pharmacy, (19th ed.) ed. A. R. Gennaro, 1995, Mack Publishing Company, Easton, Pa. and Encyclopedia of Pharmaceutical Technology, eds. J. Swarbrick and J. C. Boylan, 1988-1999, Marcel Dekker, N.Y.

In one aspect of the invention, a method of therapeutically or prophylactically treating a human patient having or at risk for having light chain (AL) amyloidosis characterized by the presence of amyloid fibrils, deposits or prefibrillar aggregates, comprises administering to the patient an effective dosage of a pharmaceutical formulation comprising: (a) an antibody comprising a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16, and which is present at a concentration of about 10 mg/mL; (b) a histidine buffer present at a concentration of about 25 mM; (c) trehalose present at a concentration of about 230 mM; (d) polysorbate 20 present at a concentration of about 0.2 g/L; and (e) a pH of about 6.5. In such a method, the dosage is typically from about 0.5 mg/kg to about 30 mg/kg of the antibody (*e.g.*, about 0.5 mg/kg to about 8 mg/kg, or about 8 mg/kg to about 30 mg/kg) administered intravenously or subcutaneously at a frequency of from about weekly to about quarterly (*e.g.*, once every 28 days).

II.C. Combinational Drug Therapy Treatment Regimes

The present invention also encompasses combination therapies for treatment or prophylaxis of amyloid disease, particularly AA amyloidosis and AL amyloidosis. Such combination therapies are performed by administering an antibody formulation of the invention in conjunction with one or more second therapeutic agents, such as another therapy to treat or effect prophylaxis of AA amyloidosis or AL amyloidosis, as the case may be. Combination therapy according to the invention may also be performed in conjunction with a second therapy is used to treat or effect prophylaxis of a disease or condition associated with amyloid disease, such as an inflammatory disease, a chronic microbial infection, a neoplasm (including malignant neoplasms), an inherited inflammatory disease, and/or a lymphoproliferative disorder. Numerous treatments are available in commercial use, in clinical evaluation, and in pre-clinical development, any of which could be selected for use in combination with the disclosed antibody formulations. Such treatments can be one or more compounds or treatments selected from, but not limited to several major categories, namely, (i) non-steroidal anti-inflammatory drugs (NSAIDs; *e.g.*, detoprofen, diclofenac, diflunisal, etodolac, fenoprofen, flurbiprofen, ibuprofen, indomethacin, ketoprofen, meclofenameate, mefenamic acid, meloxicam, nabumeone, naproxen sodium, oxaprozin, piroxicam, sulindac, tolmetin, celecoxib, rofecoxib, aspirin, choline salicylate, salsalte, and sodium and magnesium salicylate); (ii) steroids (*e.g.*, cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisolone, prednisone, triamcinolone); (iii) DMARDs, *i.e.*, disease modifying antirheumatic drugs (*e.g.*, cyclosporine, azathioprine, methotrexate, leflunomide, cyclophosphamide, hydroxychloroquine, sulfasalazine, D-penicillamine, minocycline, and gold); (iv) recombinant proteins (*e.g.*, ENBREL® (etanercept, a soluble TNF receptor) and REMICADE® (infliximab) a chimeric monoclonal anti-TNF antibody); (v) stem cell transplantation; and/or (vi) chemotherapy. Patients with AL amyloidosis may also receive treatment regimes that include drugs or combinations of drugs often used to treat hematological malignancies, such as melphalan, prednisone, dexamethasone, lenalidomide (REVLIMID®) and proteosome inhibitors such as bortezomib (VELCADE®), and carfilzomib (KYPROLIS™), at dosages in the range of the standard of care.

The duration of the combination therapy depends on the type of amyloid disease being treated, any underlying disease associated with the amyloid disease, the age and condition of the

patient, the stage and type of the patient's disease, how the patient responds to the treatment, *etc*. A medical doctor can observe the therapy's effects closely and make any adjustments as needed. Additionally, a person having a greater risk of developing AA amyloidosis (*e.g.*, a person who is genetically predisposed or previously had an inflammatory disorder or other underlying diseases) or AL amyloidosis may receive prophylactic combination treatments to inhibit or delay the development of AA AL aggregates such as fibrils, or as maintenance therapy post-treatment.

When performing a combination therapy, the two or more drug substances are administered simultaneously or sequentially in any order, *i.e.*, a formulation of the invention is administered prior to administering a second drug substance, concurrently with a second drug substance, or subsequent to administration of a second drug substance. For example, a combination therapy may be performed by administering a first therapy prior to (*e*.g., 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks before), concomitantly with, or subsequent to (*e.g.*, 1 minute, 5 minutes, 15 minutes, 30 minutes, 45 minutes, 1 hour, 2 hours, 4 hours, 6 hours, 12 hours, 24 hours, 48 hours, 72 hours, 96 hours, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 8 weeks, or 12 weeks after) administering a second agent/therapy.

The dosage, frequency and mode of administration of each component of the combination can be controlled independently. For example, one therapeutic agent/therapy may be administered orally three times per day, while the second therapeutic agent/therapy may be administered intramuscularly once per day. Combination therapy may be given in on-and-off cycles that include rest periods. The compounds may also be admixed or otherwise formulated together such that one administration delivers both compounds. In this case, each therapeutic agent is generally present in an amount of 1-95% by weight of the total weight of the composition. Alternatively, an antibody formulation of the invention and a second therapeutic agent can be formulated separately and in individual dosage amounts. Drug combinations for treatment can be provided as components of a pharmaceutical pack.

Preferably, the disclosed combination therapies elicit a synergistic therapeutic effect, *i.e.*, an effect greater than the sum of their individual effects or therapeutic outcomes. Measurable therapeutic outcomes are described herein. For example, a synergistic therapeutic effect may be

an effect of at least about two-fold greater than sum of the therapeutic effects elicited by the single agents of a given combination, or at least about five-fold greater, or at least about ten-fold greater, or at least about twenty-fold greater, or at least about fifty-fold greater, or at least about one hundred-fold greater. A synergistic therapeutic effect may also be observed as an increase in therapeutic effect of at least 10% compared to the sum of the therapeutic effects elicited by the single agents of a given combination, or at least 20%, or at least 30%, or at least 40%, or at least 50%, or at least 60%, or at least 70%, or at least 80%, or at least 90%, or at least 100%, or more. A synergistic effect is also an effect that permits reduced dosing of therapeutic agents when they are used in combination.

EXAMPLES

The following examples have been included to illustrate modes of the invention. Certain aspects of the following examples are described in terms of techniques and procedures found or contemplated by the present co-inventors to work well in the practice of the invention. In light of the present disclosure and the general level of skill in the art, those of skill appreciate that the following examples are intended to be exemplary only and that numerous changes, modifications, and alterations may be employed without departing from the scope of the invention.

Example 1. Selection of Humanized 2A4 for the Treatment of AL amyloidosis

An IgG1, kappa isotype antibody was prepared, which is a humanized version of murine antibody 2A4. The light chain and heavy chain sequences of representative humanized 2A4 antibodies are set forth in Figures 1A-1B and 3. Nucleic acids encoding the particular humanized 2A4 antibody version 3, which amino acid sequences are shown in Figure 3, are depicted in Figures 4A-4B.

The parent monoclonal 2A4 antibody is directed against a neo-carboxy terminal epitope of human serum Amyloid A (sAA), resulting from cleavage of the native sAA molecule at amino acid residue 76. The murine antibody does not cross-react with IgGs or free light chain (LC) and it has shown broad isotype recognition of patient derived AL amyloid samples examined to date. 2A4 recognizes multiple forms of AL light chain amyloid including soluble multimer and

insoluble deposits. In addition, the antibody has been shown to promote regression of amyloidoma in a mouse xenograft model. The light chain and heavy chain sequences of murine 2A4 antibody are set forth in Figure 2.

Example 2. Dose Determination for Humanized 2A4 Antibody

Nonclinical studies in the TRIAD mouse model and the cynomolgus monkey have utilized doses of 4 and 40 mg/kg in the mouse and 10, 50, and 100 mg/kg in the monkey. Conversion to the Human Equivalent Dose (HED) on a mg/kg basis (most appropriate conversion for monoclonal antibodies due to their restriction to the vascular space) gives HEDs of 0.32 and 3.2 for the mouse and 3.2, 16, and 32 for the monkey. Based on currently available data, the NOAEL in both species is expected to be the highest dose administered. Using a mouse HED (most sensitive species due to dosing limitations) of 3.2 and a 10X safety factor, the MRSD for first in man dosing would be approximately 0.32 mg/kg. Based upon animal studies, administration to humans is begun with a dose of 0.5 mg/kg.

Example 3. Preparation of the Expression Vector

For generation of the final h2A4 IgG1 HC vector the variable region of the heavy chain was isolated by PCR using the plasmid CET1019AS-hygro-h2A4VH3-Sce 4.23.07 as template. Primers used for the amplification introduced at the 5′ end of the fragments an *Mfe*I restriction site and at the 3′ end a *Blp*I restriction site for subcloning. The variable region was cloned into the *Mfe*I and *Bam*HI digested eukaryotic expression vector pBI-61, which contains the genomic constant regions of human IgG1 of G1m(3) allotype. The resulting recombinant expression vector pBI-61/2A4 IgG1-REM is 9,015 base pairs in size and carries the selectable marker dihydrofolate reductase (DHFR) from hamster under the control of the DHFR promoter and polyadenylation signal. This vector also contains the beta-lactamase gene for selection in *E. coli* as well as origins of replication for *E. coli* (ColE1 ori), SV40 (SV40 ori) and filamentous phage f1 (f1 ori). Expression of the HC is driven by the immediate early promoter/enhancer region from human cytomegalovirus (CMV) combined with a transcription enhancing element (TE) derived from the hamster genome. For transcript termination and stabilization the

polyadenylation signal from hamster growth hormone is used and for enhancement of transcription a non-coding sequence derived from the hamster genome (TE).

Using the plasmid CET1019AS-hu2A4VL3-hck-puro-Sce 4.19.07 as template the variable region of the h2A4 LC was isolated by PCR introducing at the 5′ end of the fragments an *Sgr*AI restriction site and at the 3′ end a *Kpn*I restriction site for subcloning into the final eukaryotic expression vector pBI-60 digested with the same restriction enzymes. This vector contains the genomic constant region of a human kappa chain. The resulting recombinant expression vector pBI-60/2A4 LC is 7,144 base pairs in size and contains the selectable marker neomycin phosphotransferase mutant, which confers resistance to geneticin, under the control of the SV40 promoter. For transcript termination the polyadenylation signal from *Herpes simplex* thymidine kinase is used. This vector also contains the beta-lactamase gene for selection in *E. coli* as well as origins of replication for *E. coli* (ColE1 ori) and filamentous phage f1 (f1 ori). Expression of the LC is driven by the immediate early promoter/enhancer region from human cytomegalovirus (CMV) combined with a transcription enhancing element (TE) derived from the hamster genome. For transcript termination and stabilization the polyadenylation signal from hamster growth hormone is used and for enhancement of transcription a non-coding sequence derived from the hamster genome (TE).

Example 4. Production of Humanized 2A4 Antibody (Pool-Derived Material)

Humanized 2A4 was produced in Chinese Hamster Ovary (CHO) cells, grown in chemically defined media without any bovine-derived components. Antibody was pooled from stable transfected cells from which the production cell line was ultimately derived. The pool-derived material was purified by protein A-affinity chromatography. This material was used for human tissue cross-reactivity studies and for a single dose pharmacokinetic (PK) study in cynomolgus monkeys. The formulation of the humanized 2A4 antibody is 10 mg/mL antibody, 25 mM L-Histidine/L-Histidine HCl monohydrate, 230 mM Trehalose dehydrate, 0.02% (w/v) Polysorbate (TWEEN®) 20, pH = 6.5.

Example 5. Production of Humanized 2A4 Antibody (Clone-Derived Material)

A single CHO cell clone was isolated from cell pools as described in Example 3, and was used to establish the Master Cell Bank (MCB) without any bovine materials. Humanized 2A4 for nonclinical studies was manufactured at 80 L scale using the same cell cultivation and purification processes (except scale-up modifications) as the GMP clinical version of humanized 2A4 (2,000 L scale). Material from the 2,000 L scale production may also be used in nonclinical studies.

Example 6. Process of Manufacturing Humanized 2A4 Antibody

Vial Thaw & Inoculum Expansion. Cells from the MCB are thawed and transferred into an appropriate cell culture flask. The cells are incubated at approximately 37°C. The thawed culture is propagated for one to four days (first passage after cell thaw). For sub-cultivation, an aliquot of a grown cell culture (and a defined volume of pre-warmed, 0.22 µm or less filtered inoculum medium) is used to reach a seed density of approximately 0.1 - 0.5 x 10^6 cells/mL in standard cell culture vessels of approximately 0.02 L to 1 L working volume. As an example, the first passages can be done in 0.125 L or 0.25 L or 0.5 L vessels, followed by passages in 1 L vessels. A stock culture can be initiated at this cultivation stage. For preparation of inoculum cultures for individual production fermenters, aliquots of the stock cultures are expanded to generate cultures with up to 25 L volume. Typically, the cell culture is scaled up from 1 L cultures to 2 or more 1 L or 2 L cultures, then to 2 or more 2 L or 3 L cultures and finally to 2 or more cultures with up to 25 L culture volume per vessel. Grown cell suspensions from several vessels can be pooled and used to inoculate the 80 L bioreactor. Shake flasks, T-flasks, spinner flasks and bags can be used as standard cell culture vessels for the above cultivation steps.

Seed Cultures in Bioreactors. Before inoculation with cells, 0.22 µm or less filtered growth medium is added to the bioreactors. The content of the filled bioreactors is warmed to approximately 37°C and maintained at this temperature throughout incubation of the cells. Cells from the inoculum cultures are transferred into the pre-warmed medium. The initial cell density is targeted within the range of 0.1 - 0.5 x 10^6 cells/mL. The cells are grown in an 80 L bioreactor and subsequently in a 400 L bioreactor. Cells are subcultivated approximately every two to four days. At this stage, cells may be transferred to another vessel of the same or larger volume.

Typically, the cell culture is scaled up from 1 x 80 L bioreactor culture to 1 x 400 L culture. To initiate the production phase, the cells are transferred from the grown 400 L cell suspension to the production bioreactor of approximately 2,000 L working volume.

Production culture in 2,000 L Bioreactor. Before inoculation with cells, 0.22 µm or less filtered production medium is added to the production bioreactor. The content of the filled production bioreactor is warmed to approximately 37°C and maintained at this temperature throughout incubation of the cells. The initial cell density in the production phase is targeted within the range of 0.1 - 0.5 x 10^6 cells/mL. The production bioreactor is run in a fed batch mode. To support the production of antibody and to prolong culture duration, a nutrient feed medium is added during the production stage. The point at which to start feeding is determined either by culture time or by cell density. As needed, a glucose solution and / or glutamine solution can be added during the production stage to avoid depletion of these substances during the production period. The run time of the 2,000 L production bioreactor is typically 8 to 14 days. Pre-harvest samples are tested for sterility, mycoplasma, and adventitious virus in vitro.

Harvest and Clarification. After 8 to 14 days of cultivation in the production phase, the cell culture fluid is separated from the cells. After pre-harvest sampling and prior to harvest, the pH and the temperature of the culture can be adjusted to facilitate removal of cells, debris and particles during harvest. To remove the cells, the culture is passed through a centrifugation plus dead-end filtration unit. The cells are centrifuged and / or retained by the membranes. The harvested culture fluid is passed through filters of 0.22 µm pore size or less and collected in an appropriate container. Residual culture fluid can be removed from the harvest system by flushing with Phosphate Buffered Saline (PBS) to recover residual product from the harvest system. The resulting recovered product amount is collected together with the harvested culture fluid to form the harvest pool, also called harvested cell-free culture fluid (HCCF). The pH and temperature of the HCCF can be adjusted to facilitate the subsequent downstream processing steps.

Purification. The antibody is purified from the HCCF by a series of steps involving affinity chromatography, acid treatment, depth filtration, anion exchange chromatography, cation exchange chromatography, nanofiltration and ultra-/diafiltration, several of which may be performed in several cycles. To remove contaminants the affinity chromatography process step

specifically binds the antibody product. The HCCF is applied to the chromatography column packed with the MabSelect matrix. The matrix binds antibody at neutral pH, while contaminants appear in the flow through and are removed. The column is eluted in a step elution with a 100 mM acetic acid/sodium acetate solution at pH 3.5. To inactivate potential viral contaminants, the antibody solution is incubated at room temperature for a minimum of 60 minutes at pH 3.5 ± 0.1. After incubation the acid treated pool is adjusted to pH 7.2 using a 2 M Trometamol solution and subjected to depth filtration for clarification. For anion exchange chromatography, the depth filtered product pool is adjusted to a conductivity ≤7 mS/cm with Water for Injection (WFI). The adjusted pool is applied to a chromatography column packed with Q Sepharose FF resin. The antibody passes through the anion exchange matrix unbound. The flow through is monitored and the antibody containing fraction is collected based on absorbance measurement. For cation exchange chromatography, the product pool is adjusted to a pH of 5.5 ± 0.1 by addition of acetic acid up to a conductivity of ≤7.5 mS/cm with WFI. The adjusted product pool is applied onto a chromatography column packed with SP Sepharose FF cation exchange resin. This chromatography step is performed in a bind-elute mode. The antibody binds to the cation exchange matrix. The column is eluted in a step elution with a 100 mM acetic acid/sodium acetate and 138.5 mM sodium chloride solution at pH 5.5. Potential viral contaminants are removed by passing the antibody solution through a 0.1 µm prefilter and a Planova 20N nanofilter at a maximum pressure of 1 bar differential pressure of the Planova 20N nanofilter. During ultrafiltration/diafiltration (UF/DF), the product is concentrated to the target concentration, and the buffer is exchanged with the formulation buffer. Concentration and diafiltration is performed using ultrafiltration membranes having a cut-off of approximately 30 kD. The material is processed by concentrating the product to 30-100 mg/mL. The 30 kD pool is then diafiltered with a solution of 25 mM L-Histidine, pH 6.5 and is flushed to a concentration of about 60 – 70 mg/mL. The 30 kD pool intermediate may be stored at -40°C until formulation is performed. For formulation, the 30 kD product pool is adjusted to a solution containing 17.5 mM L-Histidine / 7.5 mM L-Histidine Hydrochloride, 230 mM Trehalose, and 0.02% (w/v) Polysorbate20, pH = 6.5. The antibody is finally diluted with formulation buffer to the desired target concentration of 10 mg/mL. The resulting drug substance is filtered through a 0.22 µm

filter to remove any potential adventitious microbial contaminants and particulate material. The drug substance can be stored frozen at -40°C until filling.

Example 7. Characterization of Drug Substance Containing Humanized 2A4 Antibody

Humanized 2A4 used for formulation is composed of two heterodimers. Each of the heterodimers is composed of a heavy polypeptide chain of ~ 50 kDa (449 amino acids) and a kappa light polypeptide chain of ~ 24 kDa (219 amino acids). The antibody protein has a humanized amino acid sequence with a total molecular mass of approximately 147 kDa. The four polypeptide chains of the antibody molecule are linked together by disulfide bonds. Each heavy polypeptide chain contains one consensus sequence for N-linked glycosylation, which is occupied (positions 299 to 301, highlighted in bold and underlining in Figure 1A). There are two binding sites for the serum amyloid A epitope per antibody molecule.

A competitive binding ELISA has been established to measure binding of humanized 2A4 to its antigen (CGGHEDT (SEQ ID NO: 17) when conjugated to Ovalbumin) compared to the reference standard.

Example 8. Humanized 2A4 Drug Substance Components and Composition

The humanized 2A4 drug substance (100 mg/vial) for clinical use is a sterile liquid dosage form consisting of a 10 mL fill in a 25 mL vial (20R). The nonclinical humanized 2A4 drug substance (200 mg/vial) is 20 mL fill in a 25 mL vial (20R). The nonclinical and clinical formulations of humanized 2A4 are provided in Table 1. The final formulation of the humanized 2A4 drug substance has a density of 1.034 g/mL at 20°C and a pH of 6.5.

Table 1. Composition of Nonclinical and Clinical Humanized 2A4 Drug Substance

Component	Function	Concentration (g/L)	Nominal amount (mg/vial)	
			Nonclinical Vial Size = 25 mL (20R)	Clinical Vial Size = 25 mL (20R)
Humanized 2A4 drug substance	Active Substance	10	200	100
L-Histidine	Buffer component	2.72	54.4	27.2
L-Histidine HCl monohydrate	Buffer component	1.57	31.4	15.7
Trehalose dihydrate	Tonicity agent	87.02	1,740.4	870.2
Polysorbate (TWEEN®) 20	Surfactant	0.20	4.0	2.0
Water for Injection (WFI)	Solvent	---	Add WFI to a total volume of 20 mL	Add WFI to a total volume of 10 mL

Example 9. Batch Formula for Drug Product (100mg/ml vial)

A formula was designed for a 2,600 vial batch of drug product as provided in Table 2.

Table 2. Batch Formula for 2,600 Vials

Ingredient	Grade	Quantity per Batch
Humanized 2A4 antibody	-	260.0 g
L-Histidine	USP, Ph. Eur.	70.72 g
L-Histidine HCl monohydrate	Ph. Eur.	40.82 g
Trehalose dehydrate	USP/NF, Ph. Eur.	2,262.52 g
Polysorbate 20	USP/NF, Ph. Eur.	5.20 g

Example 10. Lyophilization

A Hof Com 26041 freeze dryer was used to lyophilize the formulated humanized 2A4 drug substance over a period of approximately 86 hours with the pressure regulated by an MKS control system (MKS Instruments) with N_2 injection according to the program set forth in Table

3. The endpoint was detected by Pirani signal. During the drying mode, the vials stand directly on the shelves without lyo plates. The nitrogen backfill is at approximately 600 mbar with pharma grade, sterile N_2. The vials were then closed and sotred at 5°C within the freeze dryer. The final drug product is stored at 2-8°C, protected from light. The process should yield a white to yellowish lyo cake.

Table 3 summarizes the program for the lyophilization of humanized 2A4 drug substance.

Table 3. Lyophilization Steps

Step	Step No.	Time [hh:mm]	Shelf temperature [°C]	Vacuum MKS [mbar]
Loading	01	00:01	5	off
Freezing	02	00:15	5	off
	03	00:05	2	off
	04	02:00	2	off
	05	01:05	-50	off
	06	02:30	-50	off
Primary Drying	07	00:05	-50	0.10
	08	00:40	-10	0.10
	09	55:00	-10	0.10
Secondary Drying	10	04:30	30	0.10
	11	20:00	30	0.10
Total Time		86:11		

Example 11. Reconstitution of Lyophilized Drug Product

Prior to application, the lyophlisate has to be reconstituted with sterilized water for injection. The reconstitution of h2A4 vials has been performed according to the following procedure under laminar air-flow. The complete flip-off-cap of the respective product vial was removed. The rubber-stopper was also removed. The solvent was added by pipetting the necessary volume (2x 5mL WFI using a piston pipette). When performing this action, it was ensured that the solvent was added slowly to the lyophilized product. The vials were carefully swirled (not shaken), until the lyophilized product was completely dissolved. The solution was made homogenous by carefully rotating the vial end-over-end. The dissolved material was aliquoted according to table 1 and stored at -70°C until analysis

Example 12. Clinical Assessment of Humanized 2A4 Drug Substance

A clinical trial is designed to determine a maximum tolerated dose (MTD) and/or the Phase 2 recommended dose (P2RD) of humanized 2A4 drug substance in subjects with AL amyloidosis. Dosing will begin at 0.5 mg/kg and escalate to a high of 30 mg/kg or 2500 mg total (whichever is lower). Initially, humanized 2A4 drug substance will be given intravenously as a single agent every 28 days until progression of organ function or unacceptable treatment related toxicity or withdraw of consent. If the half-life ($t_{1/2}$) of humanized 2A4 drug substance from the initial doses suggests that a different dosing schedule would be more appropriate (*e.g.*, every two weeks or an alternate, less frequent schedule than once every 28 days), dosing in subsequent cohorts may be modified using an alternative dosing schedule.

CLAIMS

1. A lyophilized formulation of an antibody, comprising

(a) a humanized antibody or antigen binding fragment thereof, comprising:

(i) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 6, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11; or

(ii) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 12, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11;

(b) L-histidine;

(c) L-histidine HCl monohydrate;

(d) trehalose; and

(e) polysorbate 20,

in such amounts, which upon reconstitution yield an aqueous solution wherein:

(i) the antibody or antigen binding fragment thereof is present at a concentration of about 10 mg/mL;

(ii) a histidine buffer is present at a concentration of about 25 mM;

(iii) the trehalose is present at a concentration of about 230 mM;

(iv) the polysorbate 20 is present at a concentration of about 0.2 g/L; and

wherein the aqueous solution has a pH of about 6.5.

2. The lyophilized formulation of claim 1, comprising:

(a) about 100 mg of the antibody or antigen binding fragment thereof;

(b) about 27.2 mg L-histidine;

(c) about 15.7 mg L-histidine HCl monohydrate;

(d) about 870.2 mg trehalose dihydrate; and

(e) about 2 mg polysorbate 20.

3. A lyophilized formulation of an antibody, comprising

(a) a humanized antibody or antigen binding fragment thereof, comprising:

(i) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 6, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11; or

(ii) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 12, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11;

(b) L-histidine;

(c) L-histidine HCl monohydrate;

(d) trehalose; and

(e) polysorbate 20,

in such amounts, which upon reconstitution yield an aqueous solution wherein:

(i) the antibody or antigen binding fragment thereof is present at a concentration of about 50 mg/mL;

(ii) a histidine buffer is present at a concentration of about 25 mM;

(iii) the trehalose is present at a concentration of about 230 mM;

(iv) the polysorbate 20 is present at a concentration of about 0.2 g/L; and

wherein the aqueous solution has a pH of about 6.5.

4. The lyophilized formulation of claim 3, comprising:

(a) about 500 mg of the antibody or antigen binding fragment thereof;

(b) about 27.2 mg L-histidine;

(c) about 15.7 mg L-histidine HCl monohydrate;

(d) about 870.2 mg trehalose dihydrate; and

(e) about 2 mg polysorbate 20.

5. The lyophilized formulation of any one of claims 1-4, wherein the antibody or antigen binding fragment thereof comprises a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 6, 7, and 8, and a heavy chain variable region comprising three CDRs set forth as SEQ ID NOs: 9, 10, and 11.

6. The lyophilized formulation of any one of claims 1-4, wherein the antibody or antigen binding fragment thereof comprises a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 12, 7, and 8, and a heavy chain variable region comprising three CDRs set forth as SEQ ID NOs: 9, 10, and 11.

7. The lyophilized formulation of any one of claims 1-4, wherein the antibody or antigen binding fragment thereof comprises a light chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 4 and a heavy chain variable region comprising an amino acid sequence set forth as SEQ ID NO: 5.

8. The lyophilized formulation of any one of claims 1-4, wherein the antibody or antigen binding fragment thereof comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16.

9. The lyophilized formulation of any one of claims 1-4, wherein the antibody or antigen binding fragment thereof comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as SEQ ID NO: 15.

10. A nucleic acid comprising a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 13.

11. The nucleic acid of claim 10 comprising the nucleotide sequence of SEQ ID NO: 19.

12. The nucleic acid of claim 11 comprising the nucleotide sequence of SEQ ID NO: 20.

13. A nucleic acid comprising a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as any one of SEQ ID NOs: 14-16.

14. The nucleic acid of claim 13 comprising a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 15.

15. The nucleic acid of claim 14 comprising the nucleotide sequence of SEQ ID NO: 22.

16. The nucleic acid of claim 15 comprising the nucleotide sequence of SEQ ID NO: 23.

17. A vector comprising the nucleic acid of any one of claims 10-16.

18. A vector comprising the nucleic acids of claims 10 and 14.

19. A vector comprising the nucleic acids of claims 11 and 15.

20. A vector comprising the nucleic acids of claims 12 and 16.

21. A host cell having stably incorporated into its genome the nucleic acid of any one of claims 10-16.

22. The host cell of claim 21, wherein the host cell is a CHO cell.

23. The host cell of claim 21 having stably incorporated into its genome a nucleic acid comprising the nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 13 and a nucleic acid comprising the nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as any one of SEQ ID NOs: 14-16.

24. The host cell of claim 21 having stably incorporated into its genome a nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO: 19 and a nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO: 22.

25. The host cell of claim 21 having stably incorporated into its genome a nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO: 20 and a nucleic acid comprising the nucleotide sequence set forth as SEQ ID NO: 23.

26. The host cell of any one of claims 23-25, wherein the host cell is a CHO cell.

27. A method of making a lyophilized formulation comprising:

(a) culturing mammalian cells having stably incorporated into their genome one or more nucleic acids encoding an antibody or antigen binding fragment thereof so that the cells secrete the antibody or antigen binding fragment thereof into the cell culture media, wherein the antibody or antigen binding fragment thereof comprises:

(i) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 6, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11, or

(ii) a light chain variable region comprising three CDRs set forth as SEQ ID NOs: 12, 7, and 8 and a heavy chain variable region comprising three CDRS set forth as SEQ ID NOs: 9, 10, and 11;

(b) purifying the antibody or antigen binding fragment thereof from the cell culture media; and

(c) preparing the lyophilized formulation of any one of claims 1-9.

28. The method of claim 27, wherein the nucleic acid encoding the antibody or antigen binding fragment thereof comprises a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 13, and wherein the nucleic acid encoding the heavy chain of the antibody or antigen binding fragment thereof comprises a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as any one of SEQ ID NOs: 14-16.

29. The method of claim 28, wherein the nucleic acid encoding the light chain of the antibody or antigen binding fragment thereof comprises a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 13, and wherein the

nucleic acid encoding the heavy chain of the antibody or antigen binding fragment thereof comprises a nucleotide sequence encoding a polypeptide consisting of an amino acid sequence set forth as SEQ ID NO: 15.

30. The method of claim 28, wherein the nucleic acid encoding the light chain of the antibody or antigen binding fragment thereof comprises the nucleotide sequence of SEQ ID NO: 19, and wherein the nucleic acid encoding the heavy chain of the antibody or antigen binding fragment thereof comprises the nucleotide sequence of SEQ ID NO: 22.

31. The method of claim 30, wherein the nucleic acid encoding the light chain of the antibody or antigen binding fragment thereof comprises the nucleotide sequence of SEQ ID NO: 20, and wherein the nucleic acid encoding the heavy chain of the antibody or antigen binding fragment thereof comprises the nucleotide sequence of SEQ ID NO: 23.

32. The method of any one of claims 27-31, further comprising evaluating at least one property of the antibody or antigen binding fragment thereof in the formulation selected from the group consisting of the physical stability, chemical stability and biological activity.

33. The method of any one of claims 27-32, wherein the mammalian cells are CHO cells.

34. Use of a lyophilized formulation according to any one of claims 1-9 for the manufacture of a medicament for the treatment of amyloidosis in a human patient characterized by the presence of amyloid protein fibrils.

35. The use of claim 34, wherein the human patient has amyloid A amyloidosis characterized by the presence of amyloid A protein fibrils.

36. The use of claim 34, wherein the human patient has AL amyloidosis characterized by the presence of amyloid light chain-type protein fibrils.

37. The use of claim 36, wherein the AL amyloidosis is associated with a dyscrasia of the B lymphocyte lineage.

38. The use of claim 37, wherein the dyscrasia is a malignancy.

39. The use of claim 38, wherein the malignancy is multiple myeloma.

40. The use of any one of claims 34-39, wherein the medicament is to be administered with at least one second drug selected from the group consisting of melphalan, bortezomib, lenolidomide and carfilzomib.

41. The use of claim 40, wherein the at least one second drug is bortezomib.

42. The use of any one of claims 34-41, wherein the medicament is to be administered to the human patient at a frequency of daily to annually.

43. The use of any one of claims 34-42, wherein the medicament is to be administered to the human patient once every twenty-eight days.

44. The use of any one of claims 40-43, wherein the at least one second drug is to be administered to the human patient at a frequency of daily to annually.

45. The use of any one of claims 40-44, wherein the at least one second drug is to be administered to the human patient once every seven days.

46. The use of any one of claims 34-45, wherein the medicament is to be administered to the human patient in a dose ranging from about 0.5 mg of the antibody per kg body weight of the human patient to about 30 mg of the antibody per kg of body weight of the human patient.

47. The use of any one of claims 34-46, wherein the medicament is to be administered to the human patient in a dose ranging from about 20 mg of the antibody per kg body weight of the human patient to about 30 mg of the antibody per kg of body weight of the human patient.

48. The use of any one of claims 34-47, wherein the medicament is to be administered to the human patient in a dose of about 25 mg of the antibody per kg body weight of the human patient.

49. The use of any one of claims 34-48, wherein the medicament is to be administered to the human patient in a dose ranging from about 10 mg of the antibody to about 5000 mg of the antibody.

50. The use of any one of claims 34-49, wherein the medicament is to be administered to the human patient in a dose ranging from about 30 mg of the antibody to about 2500 mg of the antibody.

51. A lyophilized formulation according to any one of claims 1-9 for use in the manufacture of a medicament for the treatment of amyloidosis in a human patient characterized by the presence of amyloid protein fibrils.

52. The lyophilized formulation of claim 51, wherein the human patient has amyloid A amyloidosis characterized by the presence of amyloid A protein fibrils.

53. The lyophilized formulation of claim 51, wherein the human patient has AL amyloidosis characterized by the presence of amyloid light chain-type protein fibrils.

54. The lyophilized formulation of claim 53, wherein the AL amyloidosis is associated with a dyscrasia of the B lymphocyte lineage.

55. The lyophilized formulation of claim 54, wherein the dyscrasia is a malignancy.

56. The lyophilized formulation of claim 55, wherein the malignancy is multiple myeloma.

57. The lyophilized formulation of any one of claims 51-56, wherein the medicament is to be administered with at least one second drug selected from the group consisting of melphalan, bortezomib, lenolidomide and carfilzomib.

58. The lyophilized formulation of claim 57, wherein the at least one second drug is bortezomib.

59. The lyophilized formulation of any one of claims 51-58, wherein the medicament is to be administered to the human patient at a frequency of daily to annually.

60. The lyophilized formulation of any one of claims 51-59, wherein the medicament is to be administered to the human patient once every twenty-eight days.

61. The lyophilized formulation of any one of claims 57-60, wherein the at least one second drug is to be administered to the human patient at a frequency of daily to annually.

62. The lyophilized formulation of any one of claims 57-61, wherein the at least one second drug is to be administered to the human patient once every seven days.

63. The lyophilized formulation of any one of claims 51-62, wherein the medicament is to be administered to the human patient in a dose ranging from about 0.5 mg of the antibody per kg body weight of the human patient to about 30 mg of the antibody per kg of body weight of the human patient.

64. The lyophilized formulation of any one of claims 51-63, wherein the medicament is to be administered to the human patient in a dose ranging from about 20 mg of the antibody per kg body weight of the human patient to about 30 mg of the antibody per kg of body weight of the human patient.

65. The lyophilized formulation of any one of claims 51-64, wherein the medicament is to be administered to the human patient in a dose of about 25 mg of the antibody per kg body weight of the human patient.

66. The lyophilized formulation of any one of claims 51-65, wherein the medicament is to be administered to the human patient in a dose ranging from about 10 mg of the antibody to about 5000 mg of the antibody.

67. The lyophilized formulation of any one of claims 51-66, wherein the medicament is to be administered to the human patient in a dose ranging from about 30 mg of the antibody to about 2500 mg of the antibody.

68. A pharmaceutical product, comprising:

(a) a vial comprising an antibody, L-histidine, trehalose and polysorbate 20 in powder form;

(b) instructions for reconstitution of the antibody; and

(c) instructions for preparing the reconstituted antibody for infusion,

wherein:

(i) the antibody comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16;

(ii) the vial comprises about 100 mg of the antibody, about 27.2 mg L-histidine, about 15.7 mg L-histidine HCl monohydrate, about 870.2 mg trehalose dehydrate, and about 2 mg polysorbate 20; and

(ii) the reconstitution instructions require reconstitution with water for injection to an extractable volume of 10 mL.

69. A pharmaceutical product, comprising:

(a) a vial comprising an antibody, L-histidine, trehalose and polysorbate 20 in powder form;

(b) instructions for reconstitution of the antibody; and

(c) instructions for preparing the reconstituted antibody for infusion,

wherein:

(i) the antibody comprises a light chain comprising an amino acid sequence set forth as SEQ ID NO: 13 and a heavy chain comprising an amino acid sequence set forth as any one of SEQ ID NOs: 14-16;

(ii) the vial comprises 500 mg of the antibody, 27.2 mg L-histidine, 15.7 mg L-histidine HCl monohydrate, 870.2 mg trehalose dehydrate, and 2 mg polysorbate 20; and

(iii) the reconstitution instructions require reconstitution with water for injection to an extractable volume of 10 mL.