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(54) Title: ANTI-TRANSTHYRETIN ANTIBODIES

	10	20	30	40	
m5A1vH	EVKL	VESGGGLV	RPGGSLKLS	CAASGFTFS	NYAMSVVROTPE 42
3LS4 H St.pro	EVKL	VESGGGLV	KPGGSLKLS	CAASGFTFNNYVMV	WLROTPE 42
AGP01680	EVQL	VESGGGLIQ	PGGSLRLS	CAASGFTVSS	SNYMSVVRQAPG 42
Hu5A1vHv1	EVQL	VESGGGLIQ	PGGSLRLS	CAASGFTFS	NYAMSVVVRQAPG 42
Hu5A1vHv2	EVQL	VESGGGLIQ	PGGSLRLS	CAASGFTFS	NYAMSVVVRQAPG 42
	50	60	70	80	
m5A1vH	KRL	EWVASSIS	SGGSTYY	PDSVKGRFTIS	RDNARNILYLQMS 84
3LS4 H St.pro	KRL	EWVASSIS	RGGSTYY	PDSVKGRFTIS	RDNARNILYLQMS 84
AGP01680	KGL	EWVSVI	YSGGSTYY	ADSVKGRFTIS	RDNASKNTLYLQMS 84
Hu5A1vHv1	KGL	EWVSSIS	SGGSTYY	PDSVKGRFTIS	RDNASKNTLYLQMS 84
Hu5A1vHv2	KGL	EWVSSIS	SGGSTYY	PDSVKGRFTIS	RDNASKNTLYLQMS 84
	90	100	110		
m5A1vH	LRSE	DAMYYCV	RYYYGQY	FDWFGQGT	ALTAVSS 117 (SEQ ID NO:1)
3LS4 H St.pro	LRSE	DAMYYCV	RGTTIVAG	DVWGAGT	TVTAVSS 117 (SEQ ID NO:2)
AGP01680	LRAE	DAMYYCAR	SVVQGP	HLDWGGT	LTAVSS 117 (SEQ ID NO:3)
Hu5A1vHv1	LRAE	DAMYYCV	RYYYGQY	FDWFGQGT	LTAVSS 117 (SEQ ID NO:4)
Hu5A1vHv2	LRAE	DAMYYCAR	SVVQGP	HLDWGGT	LTAVSS 117 (SEQ ID NO:5)

FIG. 1

(57) Abstract: The invention provides antibodies that specifically bind to transthyretin (TTR). The antibodies can be used for treating or effecting prophylaxis of diseases or disorders associated with TTR accumulation or accumulation of TTR deposits (e.g., TTR amyloidosis). The antibodies can also be used for diagnosing TTR amyloidosis and inhibiting or reducing aggregation of TTR, among other applications.

## ANTI-TRANSTHYRETIN ANTIBODIES

### CROSS REFERENCE TO RELATED APPLICATION

**[0001]** This application is related to U.S. Provisional Application No. 62/109,004 filed January 28, 2015 and U.S. Provisional Application No. 62/266,555 filed December 11, 2015, each of which is incorporated by reference in its entirety for all purposes.

### REFERENCE TO A SEQUENCE LISTING

**[0002]** This application includes an electronic sequence listing in a file named 473366\_SEQLIST.TXT, created on January 28, 2016 and containing 49,257 bytes, which is hereby incorporated by reference in its entirety for all purposes.

### BACKGROUND

**[0003]** Several diseases are thought to be caused by the abnormal folding and aggregation of disease-specific proteins. These proteins can accumulate into pathologically diagnostic accumulations, known as amyloids, which are visualized by certain histologic stains. Amyloids are thought to elicit inflammatory responses and have multiple negative consequences for the involved tissues. In addition, smaller aggregates of abnormally folded protein may exist and exert cytotoxic effects.

**[0004]** Transthyretin (TTR) is one of the many proteins that are known to misfold and aggregate (*e.g.*, undergo amyloidogenesis). Transthyretin-related amyloidosis encompasses two forms of disease: familial disease arising from misfolding of a mutated or variant TTR, and a sporadic, non-genetic disease caused by misaggregation of wild-type TTR. The process of TTR amyloidogenesis can cause pathology in the nervous system and/or heart, as well as in other tissues.

### SUMMARY OF THE CLAIMED INVENTION

**[0005]** In one aspect, the invention provides antibodies that specifically bind transthyretin comprising three heavy chain CDRs and three light chain CDRs substantially from antibody 5A1. Some such antibodies comprise three Kabat heavy chain CDRs (SEQ ID NOS:6-8,

respectively) and three light CDRs (SEQ ID NOS:14, 15, and 17, respectively) of antibody 5A1. In some antibodies, the heavy chain CDR-H1 is a composite Kabat-Chothia CDR-H1 (SEQ ID NO:52). Some such antibodies are monoclonal antibodies. In another aspect, the invention provides an antibody that specifically binds transthyretin comprising a mature heavy chain variable region comprising three Kabat heavy chain CDRs, CDR-H1 of SEQ ID NO:6, CDR-H2 of SEQ ID NO:7, and CDR-H3 of SEQ ID NO:8, and a mature light chain variable region comprising three Kabat light chain CDRs, CDR-L1 of SEQ ID NO:14, CDR-L2 of SEQ ID NO:15 or SEQ ID NO:16, and CDR-L3 of SEQ ID NO:17. Some such antibodies are chimeric, humanized, veneered, or human antibodies. Some such antibodies have a human IgG1 isotype. Some such antibodies have a human IgG2 or IgG4 isotype.

**[0006]** Some such antibodies are humanized or chimeric 5A1 antibodies that specifically bind to transthyretin, wherein 5A1 is a mouse antibody characterized by a mature heavy chain variable region of SEQ ID NO:1 and a mature light chain variable region of SEQ ID NO:9.

**[0007]** In some antibodies, the humanized mature heavy chain variable region comprises the three heavy chain CDRs of 5A1 and the humanized mature light chain variable region comprises the three light chain CDRs of 5A1, except that position L55 can be C or S. In some antibodies, the humanized mature heavy chain variable region comprises the three Kabat heavy chain CDRs of 5A1 (SEQ ID NOS:6-8) and the humanized mature light chain variable region comprises the three Kabat light chain CDRs of 5A1 (SEQ ID NOS:14, 15, and 17) except that position L55 can be C or S.

**[0008]** Some such antibodies comprise a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to SEQ ID NO:4 or 5 and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to SEQ ID NO: 12 or 13.

**[0009]** In some such antibodies, at least one of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V. In some such antibodies, at least two of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V. In some such antibodies, at least three of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V. In some such antibodies, all of the following positions are occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V.

**[0010]** Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 95% identical to SEQ ID NO:4 or 5 and a mature light chain variable region having an amino acid sequence at least 95% identical to SEQ ID NO:12 or 13. Some antibodies comprise a mature heavy chain variable region having an amino acid sequence at least 98% identical to SEQ ID NO:4 or 5 and a mature light chain variable region having an amino acid sequence at least 98% identical to SEQ ID NO:12 or 13.

**[0011]** Some such antibodies comprise a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:12. Some such antibodies comprise a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:13. Some such antibodies comprise a mature heavy chain variable region of SEQ ID NO:5 and a mature light chain variable region of SEQ ID NO:12. Some such antibodies comprise a mature heavy chain variable region of SEQ ID NO:5 and a mature light chain variable region of SEQ ID NO:13.

**[0012]** In some antibodies, the antibody is an intact antibody. In some antibodies, the antibody is a binding fragment. In some such antibodies, the binding fragment is a single-chain antibody, Fab, or Fab'2 fragment.

**[0013]** In some antibodies, the mature light chain variable region is fused to a light chain constant region and the mature heavy chain variable region is fused to a heavy chain constant region. In some such antibodies, the heavy chain constant region is a mutant form of a natural human heavy chain constant region which has reduced binding to a Fcγ receptor relative to the natural human heavy chain constant region. In some such antibodies, the heavy chain constant region is of IgG1 isotype. In some such antibodies, the mature heavy chain variable region is fused to a heavy chain constant region having the sequence of SEQ ID NO:23 and/or the mature light chain variable region is fused to a light chain constant region having the sequence of SEQ ID NO:25.

**[0014]** In some antibodies, any differences in CDRs of the mature heavy chain variable region and mature light chain variable region from SEQ ID NOS:1 and 9, respectively, reside in positions H60-H65.

**[0015]** In another aspect, the invention provides a pharmaceutical composition comprising the any of the above mentioned antibodies and a pharmaceutically acceptable carrier.

**[0016]** In another aspect, the invention provides a nucleic acid encoding the heavy chain and/or light chain of any of the above mentioned antibodies. In another aspect, the invention provides a recombinant expression vector comprising such a nucleic acid. In another aspect, the invention provides a host cell transformed with such a recombinant expression vector.

**[0017]** In another aspect, the invention provides a method of humanizing an antibody, the method comprising:

- (a) selecting an acceptor antibody;
  - (b) identifying the amino acid residues of the mouse antibody to be retained;
  - (c) synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and
  - (d) expressing the nucleic acids in a host cell to produce a humanized antibody;
- wherein the mouse antibody comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO:1 and a light chain variable region having an amino acid sequence of SEQ ID NO:9

**[0017a]** In another aspect, the invention provides a method of humanizing an antibody, the method comprising:

- (a) selecting one or more acceptor antibody sequences;
- (b) identifying amino acid residues of a mouse antibody to be retained;

(c) synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and

(d) expressing the nucleic acids in a host cell to produce a humanized antibody;

wherein the mouse antibody comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO:1 and a light chain variable region having an amino acid sequence of SEQ ID NO:9.

**[0018]** In another aspect, the invention provides a method of producing a humanized, chimeric, or veneered antibody, the method comprising:

(a) culturing cells transformed with nucleic acids encoding the heavy and light chains of the antibody, so that the cells secrete the antibody; and

(b) purifying the antibody from cell culture media;

wherein the antibody is a humanized, chimeric, or veneered form of 5A1.

**[0019]** In another aspect, the invention provides a method of producing a cell line producing a humanized, chimeric, or veneered antibody, the method comprising:

(a) introducing a vector encoding heavy and light chains of an antibody and a selectable marker into cells;



(b) propagating the cells under conditions to select for cells having increased copy number of the vector;  
 (c) isolating single cells from the selected cells; and  
 (d) banking cells cloned from a single cell selected based on yield of antibody;  
 wherein the antibody is a humanized, chimeric, or veneered form of 5A1.

**[0019a]** In another aspect, the invention provides a method of producing a cell line producing a humanized, chimeric, or veneered antibody according to any aspect, embodiment or example herein, the method comprising:

(a) introducing a vector encoding heavy and light chains of the antibody and a selectable marker into cells;  
 (b) propagating the cells under conditions to select for cells having increased copy number of the vector;  
 (c) isolating single cells from the selected cells; and  
 (d) banking cells cloned from a single cell selected based on yield of antibody

**[0020]** Some such methods further comprise propagating the cells under selective conditions and screening for cell lines naturally expressing and secreting at least 100 mg/L/10<sup>6</sup> cells/24h.

**[0021]** In another aspect, the invention provides a method of inhibiting or reducing aggregation of transthyretin in a subject having or at risk of developing a transthyretin-mediated amyloidosis, comprising administering to the subject an effective regime of any of the above mentioned antibodies, thereby inhibiting or reducing aggregation of transthyretin in the subject.

**[0022]** In another aspect, the invention provides a method of inhibiting or reducing transthyretin fibril formation in a subject having or at risk of developing a transthyretin-mediated amyloidosis, comprising administering to the subject an effective regime of any of the above mentioned antibodies, thereby inhibiting or reducing transthyretin accumulation in the subject.

**[0023]** In another aspect, the invention provides a method of reducing transthyretin deposits in a subject having or at risk of developing a transthyretin-mediated amyloidosis, comprising administering to the subject an effective regime of any of the above mentioned antibodies, thereby reducing transthyretin deposits in the subject.

**[0024]** In another aspect, the invention provides a method of clearing aggregated transthyretin in a subject having or at risk of developing a transthyretin-mediated amyloidosis, comprising administering to the subject an effective regime of any of the above mentioned antibodies, thereby clearing aggregated transthyretin from the subject relative to a subject having or at risk of developing a transthyretin-mediated amyloidosis who has not received the antibody.

**[0025]** In another aspect, the invention provides a method of stabilizing a non-toxic conformation of transthyretin in a subject having or at risk of developing a transthyretin-mediated amyloidosis, comprising administering to the subject an effective regime of any of the

above mentioned antibodies, thereby stabilizing a non-toxic conformation of transthyretin in the subject.

**[0026]** In another aspect, the invention provides a method of treating or effecting prophylaxis of a transthyretin-mediated amyloidosis in a subject, comprising administering to the subject an effective regime of any of the above mentioned antibodies.

**[0027]** In another aspect, the invention provides a method of delaying the onset of a transthyretin-mediated amyloidosis in a subject, comprising administering to the subject an effective regime of any of the above mentioned antibodies.

**[0028]** In another aspect, the invention provides a method of diagnosing a transthyretin-mediated amyloidosis in a subject, comprising contacting a biological sample from the subject with an effective amount of any of the above mentioned antibodies. Some such methods further comprise detecting the binding of antibody to transthyretin, wherein the presence of bound antibody indicates the subject has a transthyretin-mediated amyloidosis. Some such methods further comprise comparing binding of the antibody to the biological sample with binding of the antibody to a control sample, whereby increased binding of the antibody to the biological sample relative to the control sample indicates the subject has a transthyretin-mediated amyloidosis.

**[0029]** In some such methods, the biological sample and the control sample comprise cells of the same tissue origin. In some such methods, the biological sample and/or the control sample is blood, serum, plasma, or solid tissue. In some such methods, the solid tissue is from the heart, peripheral nervous system, autonomic nervous system, kidneys, eyes, or gastrointestinal tract.

**[0030]** In some methods, the transthyretin-mediated amyloidosis is a familial transthyretin amyloidosis or a sporadic transthyretin amyloidosis. In some such methods, the familial transthyretin amyloidosis is familial amyloid cardiomyopathy (FAC), familial amyloid polyneuropathy (FAP), or central nervous system selective amyloidosis (CNSA). In some such methods, the sporadic transthyretin amyloidosis is senile systemic amyloidosis (SSA) or senile cardiac amyloidosis (SCA).

**[0031]** In some methods, the transthyretin-mediated amyloidosis is associated with amyloid accumulation in the heart, peripheral nervous system, autonomic nervous system, kidneys, eyes, or gastrointestinal tract of the subject.

**[0032]** In another aspect, the invention provides a method of detecting the presence or absence of transthyretin deposits in a subject, comprising contacting a biological sample from the subject suspected of comprising the amyloid accumulation with an effective amount of any of the above mentioned antibodies. Some such methods further comprise detecting the binding of antibody to transthyretin, wherein detection of bound antibody indicates the presence of transthyretin deposits. Some such methods further comprise comparing binding of the antibody to the biological sample with binding of the antibody to a control sample, whereby increased binding of the antibody to the biological sample relative to the control sample indicates the subject has a transthyretin-mediated amyloidosis. In some such methods, the biological sample and the control sample comprise cells of the same tissue origin. In some such methods, the biological sample and/or the control sample is blood, serum, plasma, or solid tissue. In some such methods, the solid tissue is from the heart, peripheral nervous system, autonomic nervous system, kidneys, eyes, or gastrointestinal tract.

**[0033]** In another aspect, the invention provides a method of determining a level of transthyretin deposits in a subject, comprising administering any of the above mentioned antibodies and detecting the presence of bound antibody in the subject. In some such methods, the presence of bound antibody is determined by positron emission tomography (PET).

### **BRIEF DESCRIPTION OF THE DRAWINGS**

**[0034]** FIG. 1 depicts an alignment of heavy chain variable regions of the mouse 5A1 antibody, mouse model antibodies, human acceptor antibodies, and humanized versions of the 5A1 antibody. The CDRs as defined by Kabat are enclosed in boxes, except that the first enclosed box is a composite of the Chothia CDR-H1 and the Kabat CDR-H1, with the Kabat CDR-H1 underlined and bolded.

**[0035]** FIG. 2 depicts an alignment of light chain variable regions of the mouse 5A1 antibody, mouse model antibodies, human acceptor antibodies, and humanized versions of the 5A1 antibody. The CDRs as defined by Kabat are enclosed

**[0036]** FIGS. 3A & 3B: FIG. 3A depicts the binding curve of murine 5A1, 6C1, 9D5, and 14G8 antibodies to ph4-treated TTR. FIG. 3B depicts the binding curve of murine 5A1, 6C1, 9D5, and 14G8 antibodies to ph4-treated or native TTR

**[0037]** FIG. 4A, 4B & 4C: FIG. 4A depicts the inhibition of TTR-Y78F fiber formation by mis-TTR antibodies. FIG. 4B depicts the inhibition of TTR-V122I fiber formation by 14G8. FIG. 4C depicts the inhibition of TTR-V122I fiber formation by a control antibody.

**[0038]** FIGS. 5A & 5B: FIG 5A depicts a densitometry analysis of a Western Blot analysis of plasma samples from patients confirmed for V30M ATTR (Sample #21, #22, #23, #24, #25, and #27) and samples from normal subjects (Sample #11, #12, #15, #18, #19, #20) using the 9D5 mis-TTR antibody. FIG. 5B depicts a densitometry analysis of a Western blot analysis of the same samples using the 5A1 mis-TTR antibody.

**[0039]** FIG. 6 depicts a MesoScale Discovery (MSD) plate assay of plasma samples from patients confirmed for V30M ATTR (Sample #21, #22, #23, #24, #25, #27) and samples from normal subjects (#11, #12, #15, #18, #19, #20) using the 6C1 antibody.

**[0040]** FIGS. 7A & 7B: FIG. 7A depicts the effect of antibody 14G8 on the uptake of F87M/L110M TTR by THP-1 cells. FIG. 7B depicts the effect of each of the mis-TTR antibodies on the uptake of V30M TTR by THP-1 cells.

### **BRIEF DESCRIPTION OF THE SEQUENCES**

**[0041]** SEQ ID NO:1 sets forth the amino acid sequence of the heavy chain variable region of the mouse 5A1 antibody.

**[0042]** SEQ ID NO:2 sets forth the amino acid sequence of the mouse heavy chain variable region structure template.

**[0043]** SEQ ID NO:3 sets forth the amino acid sequence of the heavy chain variable acceptor accession number AGP01680.

**[0044]** SEQ ID NO:4 sets forth the amino acid sequence of the heavy chain variable region of the humanized 5A1 antibody version 1 (Hu5A1VHv1).

**[0045]** SEQ ID NO:5 sets forth the amino acid sequence of the heavy chain variable region of the humanized 5A1 antibody version 2 (Hu5A1VHv2).

**[0046]** SEQ ID NO:6 sets forth the amino acid sequence of Kabat CDRH1 of the mouse 5A1 antibody.

**[0047]** SEQ ID NO:7 sets forth the amino acid sequence of Kabat CDRH2 of the mouse 5A1 antibody.

**[0048]** SEQ ID NO:8 sets forth the amino acid sequence of Kabat CDRH3 of the mouse 5A1 antibody.

**[0049]** SEQ ID NO:9 sets forth the amino acid sequence of the light chain variable region of the mouse 5A1 antibody.

**[0050]** SEQ ID NO:10 sets forth the amino acid sequence of the mouse light chain variable region structure template.

**[0051]** SEQ ID NO:11 sets forth the amino acid sequence of the light chain variable acceptor accession number BAH04766.

**[0052]** SEQ ID NO:12 sets forth the amino acid sequence of the light chain variable region of the humanized 5A1 antibody version 1 (Hu5A1VLv1).

**[0053]** SEQ ID NO:13 sets forth the amino acid sequence of the light chain variable region of the humanized 5A1 antibody version 2 (Hu5A1VLv2).

**[0054]** SEQ ID NO:14 sets forth the amino acid sequence of Kabat CDRL1 of the mouse 5A1 antibody.

- [0055]** SEQ ID NO:15 sets forth the amino acid sequence of Kabat CDRL2 of the mouse 5A1 antibody.
- [0056]** SEQ ID NO:16 sets forth the amino acid sequence of Kabat CDRL2 version 2 of the humanized mouse 5A1 antibody.
- [0057]** SEQ ID NO:17 sets forth the amino acid sequence of Kabat CDRL3 of the mouse 5A1 antibody.
- [0058]** SEQ ID NO:18 sets forth the nucleic acid sequence of the heavy chain variable region of the mouse 5A1 antibody with signal peptide.
- [0059]** SEQ ID NO:19 sets forth the amino acid sequence of the heavy chain variable region of the mouse 5A1 antibody with signal peptide.
- [0060]** SEQ ID NO:20 sets forth the nucleic acid sequence of the light chain variable region of the mouse 5A1 antibody with signal peptide.
- [0061]** SEQ ID NO:21 sets forth the amino acid sequence of the light chain variable region of the mouse 5A1 antibody with signal peptide.
- [0062]** SEQ ID NO:22 sets forth the amino acid sequence of an exemplary IgG1 heavy chain constant region.
- [0063]** SEQ ID NO:23 sets forth the amino acid sequence of an exemplary IgG1 G1m3 heavy chain constant region.
- [0064]** SEQ ID NO:24 sets forth the amino acid sequence of an exemplary IgG1 G1m3 heavy chain constant region.
- [0065]** SEQ ID NO:25 sets forth the amino acid sequence of an exemplary light chain constant region with N-terminal Arginine.
- [0066]** SEQ ID NO:26 sets forth the amino acid sequence of an exemplary light chain constant region without N-terminal Arginine.

**[0067]** SEQ ID NO:27 sets forth the amino acid sequence of the heavy chain region of the humanized 5A1 antibody version 1.

**[0068]** SEQ ID NO:28 sets forth the amino acid sequence of the heavy chain region of the humanized 5A1 antibody version 2.

**[0069]** SEQ ID NO:29 sets forth the amino acid sequence of the light chain region of the humanized 5A1 antibody version 1.

**[0070]** SEQ ID NO:30 sets forth the amino acid sequence of the light chain region of the humanized 5A1 antibody version 2.

**[0071]** SEQ ID NO:31 sets forth the amino acid sequence of human transthyretin set forth in accession number P02766.1 (UniProt).

**[0072]** SEQ ID NO:32 sets forth the amino acid sequence of human transthyretin set forth in accession number AAB35639.1 (GenBank).

**[0073]** SEQ ID NO:33 sets forth the amino acid sequence of human transthyretin set forth in accession number AAB35640.1 (GenBank).

**[0074]** SEQ ID NO:34 sets forth the amino acid sequence of human transthyretin set forth in accession number and ABI63351.1 (GenBank).

**[0075]** SEQ ID NO:35 sets forth the amino acid sequence of a human transthyretin epitope of residues 89-97.

**[0076]** SEQ ID NO:36 sets forth the amino acid sequence of a potential transthyretin immunogen.

**[0077]** SEQ ID NO:37 sets forth the amino acid sequence of a potential transthyretin immunogen.

**[0078]** SEQ ID NO:38 sets forth the amino acid sequence of a potential transthyretin immunogen.



- [0079]** SEQ ID NO:39 sets forth a nucleic acid sequence encoding an exemplary IgG1 G1m3 heavy chain constant region.
- [0080]** SEQ ID NO:40 sets forth a nucleic acid sequence encoding an exemplary light chain constant region with N-terminal Arginine.
- [0081]** SEQ ID NO:41 sets forth a nucleic acid sequence encoding an exemplary light chain constant region without N-terminal Arginine.
- [0082]** SEQ ID NO:42 sets forth the amino acid sequence of a heavy chain constant region signal peptide.
- [0083]** SEQ ID NO:43 sets forth a nucleic acid sequence encoding a heavy chain constant region signal peptide.
- [0084]** SEQ ID NO:44 sets forth the amino acid sequence of a light chain constant region signal peptide.
- [0085]** SEQ ID NO:45 sets forth a nucleic acid sequence encoding a light chain constant region signal peptide.
- [0086]** SEQ ID NO:46 sets forth a nucleic acid sequence encoding a mouse 5A1 variable light chain region.
- [0087]** SEQ ID NO:47 sets forth a nucleic acid sequence encoding a mouse 5A1 variable heavy chain region.
- [0088]** SEQ ID NO:48 sets forth a nucleic acid sequence encoding a heavy chain variable region of the humanized 5A1 antibody version 1 (Hu5A1VHv1).
- [0089]** SEQ ID NO:49 sets forth a nucleic acid sequence encoding a heavy chain variable region of the humanized 5A1 antibody version 2 (Hu5A1VHv2).
- [0090]** SEQ ID NO:50 sets forth a nucleic acid sequence encoding a light chain variable region of the humanized 5A1 antibody version 1 (Hu5A1VLv1).

**[0091]** SEQ ID NO:51 sets forth a nucleic acid sequence encoding a light chain variable region of the humanized 5A1 antibody version 2 (Hu5A1VLv2).

**[0092]** SEQ ID NO:52 sets forth the amino acid sequence of a composite CDR-H1 (residues 26-35) of the mouse 5A1 antibody.

## DEFINITIONS

**[0093]** Monoclonal antibodies or other biological entities are typically provided in isolated form. This means that an antibody or other biological entity is typically at least 50% w/w pure of interfering proteins and other contaminants arising from its production or purification but does not exclude the possibility that the monoclonal antibody is combined with an excess of pharmaceutically acceptable carrier(s) or other vehicle intended to facilitate its use. Sometimes monoclonal antibodies are at least 60%, 70%, 80%, 90%, 95% or 99% w/w pure of interfering proteins and contaminants from production or purification. Often an isolated monoclonal antibody or other biological entity is the predominant macromolecular species remaining after its purification.

**[0094]** Specific binding of an antibody to its target antigen means an affinity of at least  $10^6$ ,  $10^7$ ,  $10^8$ ,  $10^9$ , or  $10^{10} \text{ M}^{-1}$ . Specific binding is detectably higher in magnitude and distinguishable from non-specific binding occurring to at least one unrelated target. Specific binding can be the result of formation of bonds between particular functional groups or particular spatial fit (*e.g.*, lock and key type) whereas nonspecific binding is usually the result of van der Waals forces. Specific binding does not however necessarily imply that an antibody binds one and only one target.

**[0095]** The basic antibody structural unit is a tetramer of subunits. Each tetramer includes two identical pairs of polypeptide chains, each pair having one "light" (about 25 kDa) and one "heavy" chain (about 50-70 kDa). The amino-terminal portion of each chain includes a variable region of about 100 to 110 or more amino acids primarily responsible for antigen recognition. This variable region is initially expressed linked to a cleavable signal peptide. The variable region without the signal peptide is sometimes referred to as a mature variable region. Thus, for example, a light chain mature variable region means a light chain variable region without the

light chain signal peptide. The carboxy-terminal portion of each chain defines a constant region primarily responsible for effector function.

**[0096]** Light chains are classified as either kappa or lambda. Heavy chains are classified as gamma, mu, alpha, delta, or epsilon, and define the antibody's isotype as IgG, IgM, IgA, IgD and IgE, respectively. Within light and heavy chains, the variable and constant regions are joined by a "J" region of about 12 or more amino acids, with the heavy chain also including a "D" region of about 10 or more amino acids. *See generally, Fundamental Immunology*, Paul, W., ed., 2nd ed. Raven Press, N.Y., 1989, Ch. 7 (incorporated by reference in its entirety for all purposes).

**[0097]** An immunoglobulin light or heavy chain variable region (also referred to herein as a "light chain variable domain" ("VL domain") or "heavy chain variable domain" ("VH domain"), respectively) consists of a "framework" region interrupted by three "complementarity determining regions" or "CDRs." The framework regions serve to align the CDRs for specific binding to an epitope of an antigen. The CDRs include the amino acid residues of an antibody that are primarily responsible for antigen binding. From amino-terminus to carboxyl-terminus, both VL and VH domains comprise the following framework (FR) and CDR regions: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4. CDRs 1, 2, and 3 of a VL domain are also referred to herein, respectively, as CDR-L1, CDR-L2, and CDR-L3; CDRs 1, 2, and 3 of a VH domain are also referred to herein, respectively, as CDR-H1, CDR-H2, and CDR-H3.

**[0098]** The assignment of amino acids to each VL and VH domain is in accordance with any conventional definition of CDRs. Conventional definitions include, the Kabat definition (Kabat, *Sequences of Proteins of Immunological Interest* (National Institutes of Health, Bethesda, MD, 1987 and 1991), The Chothia definition (Chothia & Lesk, *J. Mol. Biol.* 196:901-917, 1987; Chothia *et al.*, *Nature* 342:878-883, 1989); a composite of Chothia Kabat CDR in which CDR-H1 is a composite of Chothia and Kabat CDRs; the AbM definition used by Oxford Molecular's antibody modelling software; and, the contact definition of Martin et al (bioinfo.org.uk/abs) (*see* Table 1). Kabat provides a widely used numbering convention (Kabat numbering) in which corresponding residues between different heavy chains or between different light chains are assigned the same number. When an antibody is said to comprise CDRs by a certain definition of CDRs (*e.g.*, Kabat) that definition specifies the minimum number of CDR residues present in

the antibody (*i.e.*, the Kabat CDRs). It does not exclude that other residues falling within another conventional CDR definition but outside the specified definition are also present. For example, an antibody comprising CDRs defined by Kabat includes among other possibilities, an antibody in which the CDRs contain Kabat CDR residues and no other CDR residues, and an antibody in which CDR H1 is a composite Chothia-Kabat CDR H1 and other CDRs contain Kabat CDR residues and no additional CDR residues based on other definitions.

**Table 1**  
**Conventional Definitions of CDRs Using Kabat Numbering**

Loop	Kabat	Chothia	Composite of Chothia & Kabat	AbM	Contact
L1	L24--L34	L24--L34	L24--L34	L24--L34	L30--L36
L2	L50--L56	L50--L56	L50--L56	L50--L56	L46--L55
L3	L89--L97	L89--L97	L89--L97	L89--L97	L89--L96
H1	H31--H35B	H26--H32..H34*	H26--H35B*	H26--H35B	H30--H35B
H2	H50--H65	H52--H56	H50--H65	H50--H58	H47--H58
H3	H95--H102	H95--H102	H95--H102	H95--H102	H93--H101

\*CDR-H1 by Chothia can end at H32, H33, or H34 (depending on the length of the loop). This is because the Kabat numbering scheme places insertions of extra residues at 35A and 35B, whereas Chothia numbering places them at 31A and 31B. If neither H35A nor H35B (Kabat numbering) is present, the Chothia CDR-H1 loop ends at H32. If only H35A is present, it ends at H33. If both H35A and H35B are present, it ends at H34.

**[0099]** The term “antibody” includes intact antibodies and binding fragments thereof. Typically, fragments compete with the intact antibody from which they were derived for specific binding to the target including separate heavy chains, light chains Fab, Fab', F(ab')<sub>2</sub>, F(ab)c, Dabs, nanobodies, and Fv. Fragments can be produced by recombinant DNA techniques, or by enzymatic or chemical separation of intact immunoglobulins. The term “antibody” also includes a bispecific antibody and/or a humanized antibody. A bispecific or bifunctional antibody is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding

sites (*see, e.g.,* Songsivilai and Lachmann, *Clin. Exp. Immunol.*, 79:315-321 (1990); Kostelny *et al.*, *J. Immunol.*, 148:1547-53 (1992)). In some bispecific antibodies, the two different heavy/light chain pairs include a humanized 5A1 heavy chain/light chain pair and a heavy chain/light chain pair specific for a different epitope on transthyretin than that bound by 5A1.

**[00100]** In some bispecific antibodies, one heavy chain/light chain pair is a humanized 5A1 antibody as further disclosed below and the other heavy chain/light chain pair is from an antibody that binds to a receptor expressed on the blood brain barrier, such as an insulin receptor, an insulin-like growth factor (IGF) receptor, a leptin receptor, or a lipoprotein receptor, or a transferrin receptor (Friden *et al.*, *Proc. Natl. Acad. Sci. USA* 88:4771-4775, 1991; Friden *et al.*, *Science* 259:373-377, 1993). Such a bispecific antibody can be transferred cross the blood brain barrier by receptor-mediated transcytosis. Brain uptake of the bispecific antibody can be further enhanced by engineering the bi-specific antibody to reduce its affinity to the blood brain barrier receptor. Reduced affinity for the receptor resulted in a broader distribution in the brain (*see, e.g.,* Atwal *et al.*, *Sci. Trans. Med.* 3, 84ra43, 2011; Yu *et al.*, *Sci. Trans. Med.* 3, 84ra44, 2011).

**[00101]** Exemplary bispecific antibodies can also be: (1) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu *et al.*, Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig™) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (2) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (3) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (4) a so-called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; or (5) a so-called Scorpion molecule, comprising, *e.g.*, two scFvs fused to both termini of a human Fc-region. Examples of platforms useful for preparing bispecific antibodies include BiTE (Micromet), DART (MacroGenics), Fcab and Mab2 (F-star), Fc-engineered IgG1 (Xencor) or DuoBody (based on Fab arm exchange, Genmab).

**[00102]** The term “epitope” refers to a site on an antigen to which an antibody binds. An epitope can be formed from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of one or more proteins. Epitopes formed from contiguous amino acids (also known as linear epitopes) are typically retained on exposure to denaturing solvents whereas epitopes formed by tertiary folding (also known as conformational epitopes) are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, and more usually, at least 5 or 8-10 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance. *See, e.g.*, Epitope Mapping Protocols, in *Methods in Molecular Biology*, Vol. 66, Glenn E. Morris, Ed. (1996). The epitope can be linear, such as an epitope of, for example, 2-5, 3-5, 3-9, or 5-9 contiguous amino acids from SEQ ID NO:31. The epitope can also be a conformational epitope including, for example, two or more non-contiguous segments of amino acids within residues 89-97 of SEQ ID NO:31. If an antibody is said to bind to an epitope within amino acids 89-97 of transthyretin (TTR), for example, what is meant is that the epitope is within the recited range of amino acids including those defining the outer-limits of the range. It does not necessarily mean that every amino acid within the range constitutes part of the epitope. Thus, for example, an epitope within amino acids 89-97 of TTR may consist of amino acids 89-97, 89-96, 90-96, 91-96, 92-96, 93-96, 94-96, 89-96, 89-95, 89-94, 89-93, 89-92 or 89-93, among other linear segments of SEQ ID NO:35, or in the case of conformational epitopes, non-contiguous segments of amino acids of SEQ ID NO:35.

**[00103]** Antibodies that recognize the same or overlapping epitopes can be identified in a simple immunoassay showing the ability of one antibody to compete with the binding of another antibody to a target antigen. The epitope of an antibody can also be defined X-ray crystallography of the antibody bound to its antigen to identify contact residues. Alternatively, two antibodies have the same epitope if all amino acid mutations in the antigen that reduce or eliminate binding of one antibody reduce or eliminate binding of the other. Two antibodies have overlapping epitopes if some amino acid mutations that reduce or eliminate binding of one antibody reduce or eliminate binding of the other.

**[00104]** Competition between antibodies is determined by an assay in which an antibody under test inhibits specific binding of a reference antibody to a common antigen (*see, e.g.*,

Junghans *et al.*, *Cancer Res.* 50:1495, 1990). A test antibody competes with a reference antibody if an excess of a test antibody (*e.g.*, at least 2x, 5x, 10x, 20x or 100x) inhibits binding of the reference antibody by at least 50% as measured in a competitive binding assay. Some test antibodies inhibit binding of the reference antibody by at least 75%, 90% or 99%. Antibodies identified by competition assay (competing antibodies) include antibodies binding to the same epitope as the reference antibody and antibodies binding to an adjacent epitope sufficiently proximal to the epitope bound by the reference antibody for steric hindrance to occur.

**[00105]** The term “native” with respect to the structure transthyretin (TTR) refers to the normal folded structure of TTR in its properly functioning state (*i.e.*, a TTR tetramer). As TTR is a tetramer in its natively folded form, non-native forms of TTR include, for example, misfolded TTR tetramers, TTR monomers, aggregated forms of TTR, and fibril forms of TTR. Non-native forms of TTR can include molecules comprising wild-type TTR amino acid sequences or mutations.

**[00106]** The term “misfolded” with respect to TTR refers to the secondary and tertiary structure of a TTR polypeptide monomer or multimer, and indicates that the polypeptide has adopted a conformation that is not normal for that protein in its properly functioning state. Although TTR misfolding can be caused by mutations in the protein (*e.g.*, deletion, substitution, or addition), wild-type TTR proteins can also be misfolded in diseases, exposing specific epitopes.

**[00107]** The term “pharmaceutically acceptable” means that the carrier, diluent, excipient, or auxiliary is compatible with the other ingredients of the formulation and not substantially deleterious to the recipient thereof.

**[00108]** The term “patient” includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

**[00109]** An individual is at increased risk of a disease if the subject has at least one known risk-factor (*e.g.*, genetic, biochemical, family history, and situational exposure) placing individuals with that risk factor at a statistically significant greater risk of developing the disease than individuals without the risk factor.

**[00110]** The term “biological sample” refers to a sample of biological material within or obtainable from a biological source, for example a human or mammalian subject. Such samples can be organs, organelles, tissues, sections of tissues, bodily fluids, peripheral blood, blood plasma, blood serum, cells, molecules such as proteins and peptides, and any parts or combinations derived therefrom. The term biological sample can also encompass any material derived by processing the sample. Derived material can include cells or their progeny. Processing of the biological sample may involve one or more of filtration, distillation, extraction, concentration, fixation, inactivation of interfering components, and the like.

**[00111]** The term “control sample” refers to a biological sample not known or suspected to include monomeric, misfolded, aggregated, or fibril forms of transthyretin (TTR), such as in TTR amyloid deposits. Control samples can be obtained from individuals not afflicted with a TTR amyloidosis or a specifically chosen type of TTR amyloidosis. Alternatively, control samples can be obtained from patients afflicted with TTR amyloidosis or a specifically chosen type of TTR amyloidosis. Such samples can be obtained at the same time as a biological sample thought to comprise the TTR amyloidosis or on a different occasion. A biological sample and a control sample can both be obtained from the same tissue (*e.g.*, a tissue section containing both TTR amyloid deposits and surrounding normal tissue). Preferably, control samples consist essentially or entirely of tissue free of TTR amyloid deposits and can be used in comparison to a biological sample thought to comprise TTR amyloid deposits. Preferably, the tissue in the control sample is the same type as the tissue in the biological sample (*e.g.*, cardiomyocytes in the heart).

**[00112]** The term “disease” refers to any abnormal condition that impairs physiological function. The term is used broadly to encompass any disorder, illness, abnormality, pathology, sickness, condition, or syndrome in which physiological function is impaired, irrespective of the nature of the etiology.

**[00113]** The term “symptom” refers to a subjective evidence of a disease, such as altered gait, as perceived by the subject. A “sign” refers to objective evidence of a disease as observed by a physician.



**[00114]** For purposes of classifying amino acids substitutions as conservative or nonconservative, amino acids are grouped as follows: Group I (hydrophobic side chains): met, ala, val, leu, ile; Group II (neutral hydrophilic side chains): cys, ser, thr; Group III (acidic side chains): asp, glu; Group IV (basic side chains): asn, gln, his, lys, arg; Group V (residues influencing chain orientation): gly, pro; and Group VI (aromatic side chains): trp, tyr, phe. Conservative substitutions involve substitutions between amino acids in the same class. Non-conservative substitutions constitute exchanging a member of one of these classes for a member of another.

**[00115]** Percentage sequence identities are determined with antibody sequences maximally aligned by the Kabat numbering convention. After alignment, if a subject antibody region (*e.g.*, the entire mature variable region of a heavy or light chain) is being compared with the same region of a reference antibody, the percentage sequence identity between the subject and reference antibody regions is the number of positions occupied by the same amino acid in both the subject and reference antibody region divided by the total number of aligned positions of the two regions, with gaps not counted, multiplied by 100 to convert to percentage.

**[00116]** Compositions or methods “comprising” or “including” one or more recited elements may include other elements not specifically recited. For example, a composition that “comprises” or “includes” an antibody may contain the antibody alone or in combination with other ingredients.

**[00117]** Designation of a range of values includes all integers within or defining the range, and all subranges defined by integers within the range.

**[00118]** Unless otherwise apparent from the context, the term “about” encompasses values within a standard margin of error of measurement (*e.g.*, SEM) of a stated value.

**[00119]** Statistical significance means  $p \leq 0.05$ .

**[00120]** The singular forms of the articles “a,” “an,” and “the” include plural references unless the context clearly dictates otherwise. For example, the term “a compound” or “at least one compound” can include a plurality of compounds, including mixtures thereof.

## DETAILED DESCRIPTION

### I. General

**[00121]** The invention provides antibodies that specifically bind to residues 89-97 of transthyretin (TTR). The antibodies have the capacity to bind to monomeric, misfolded, aggregated, or fibril forms of TTR. The antibodies can be used for treating or effecting prophylaxis of diseases or disorders associated with TTR accumulation or accumulation of TTR deposits (*e.g.*, TTR amyloidosis). The antibodies can also be used for diagnosing TTR amyloidosis and inhibiting or reducing aggregation of TTR, among other applications.

### II. Target Molecules

**[00122]** Transthyretin (TTR) is a 127-amino acid, 55 kDa serum and cerebrospinal fluid transport protein primarily synthesized by the liver. It has also been referred to as prealbumin, thyroxine binding prealbumin, ATTR, and TBPA. In its native state, TTR exists as a tetramer. In homozygotes, the tetramers comprise identical 127-amino-acid beta-sheet-rich subunits. In heterozygotes, the TTR tetramers are made up of variant and/or wild-type subunits, typically combined in a statistical fashion.

**[00123]** The established function of TTR in the blood is to transport *holo*-retinol binding protein. Although TTR is the major carrier of thyroxine (T<sub>4</sub>) in the blood of rodents, utilizing binding sites that are orthogonal to those used for *holo*-retinol binding protein, the T<sub>4</sub> binding sites are effectively unoccupied in humans.

**[00124]** TTR is one of at least thirty different human proteins whose extracellular misfolding and/or misassembly (amyloidogenesis) into a spectrum of aggregate structures is thought to cause degenerative diseases referred to as amyloid diseases. TTR undergoes conformational changes in order to become amyloidogenic. Partial unfolding exposes stretches of largely uncharged hydrophobic residues in an extended conformation that efficiently misassemble into largely unstructured spherical aggregates that ultimately undergo conformation conversion into cross-beta sheet amyloid structures.

**[00125]** Unless otherwise apparent from context, reference to transthyretin (TTR) or its fragments or domains includes the natural human amino acid sequences including isoforms, mutants, and allelic variants thereof. Exemplary TTR polypeptide sequences are designated by Accession Numbers P02766.1 (UniProt) (SEQ ID NO:31), AAB35639.1 (GenBank) (SEQ ID NO:32), AAB35640.1 (GenBank) (SEQ ID NO:33), and ABI63351.1 (GenBank) (SEQ ID NO:34). Residues are numbered according to Swiss Prot P02766.1, with the first amino acid of the mature protein (*i.e.*, not including the 20 amino acid signal sequence) designated residue 1. In any other TTR protein, residues are numbered according to the corresponding residues in P02766.1 on maximum alignment.

### **III. Transthyretin Amyloidosis**

**[00126]** Transthyretin (TTR) amyloidosis is a systemic disorder characterized by pathogenic, misfolded TTR and the extracellular deposition of amyloid fibrils composed of TTR. TTR amyloidosis is generally caused by destabilization of the native TTR tetramer form (due to environmental or genetic conditions), leading to dissociation, misfolding, and aggregation of TTR into amyloid fibrils that accumulate in various organs and tissues, causing progressive dysfunction. *See, e.g.*, Almeida and Saraiva, *FEBS Letters* 586:2891-2896 (2012); Ando *et al.*, *Orphanet Journal of Rare Diseases* 8:31 (2013).

**[00127]** In humans, both wild-type TTR tetramers and mixed tetramers comprised of mutant and wild-type subunits can dissociate, misfold, and aggregate, with the process of amyloidogenesis leading to the degeneration of post-mitotic tissue. Thus, TTR amyloidoses encompass diseases caused by pathogenic misfolded TTR resulting from mutations in TTR or resulting from non-mutated, misfolded TTR.

**[00128]** For example, senile systemic amyloidosis (SSA) and senile cardiac amyloidosis (SCA) are age-related types of amyloidosis that result from the deposition of wild-type TTR amyloid outside and within the cardiomyocytes of the heart. TTR amyloidosis is also the most common form of hereditary (familial) amyloidosis, which is caused by mutations that destabilize the TTR protein. The TTR amyloidoses associated with point mutations in the TTR gene include familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and the rare central nervous system selective amyloidosis (CNSA). Patients with hereditary (familial) TTR

amyloidosis are almost always heterozygotes, meaning that the TTR tetramers are composed of mutant and/or wild-type TTR subunits, generally statistically distributed. Hereditary (familial) versions of TTR amyloidosis are generally autosomal dominant and are typically earlier onset than the sporadic diseases (SSA and SCA).

**[00129]** There are over 100 mutations in the gene encoding TTR that have been implicated in the autosomal dominant disorders FAP and FAC. *See, e.g.,* US 2014/0056904; Saraiva, *Hum. Mutat.* 17(6):493-503 (2001); Damas and Saraiva, *J. Struct. Biol.* 130:290-299; Dwulet and Benson, *Biochem. Biophys. Res. Commun.* 114:657-662 (1983). These amyloid-causing mutations are distributed throughout the entire molecule of TTR. Generally, the more destabilizing the mutant subunits are to the TTR tetramer structure, the earlier the onset of amyloid disease. The pathogenic potential of a TTR variant is generally determined by a combination of its instability and its cellular secretion efficiency. The initial pathology caused by some TTR variants comes from their selective destruction of cardiac tissue, whereas that from other TTR variants comes from compromising the peripheral and autonomic nervous system. The tissue damage caused by TTR amyloidogenesis appear to stem largely from the toxicity of small, diffusible TTR aggregates, although accumulation of extracellular amyloid may contribute and almost certainly compromises organ structure in the late stages of the TTR amyloidosis.

**[00130]** TTR amyloidosis presents in many different forms, with considerable phenotypic variation across individuals and geographic locations. For example, TTR amyloidosis can present as a progressive, axonal sensory autonomic and motor neuropathy. TTR amyloidosis can also present as an infiltrative cardiomyopathy.

**[00131]** The age at onset of disease-related symptoms varies between the second and ninth decades of life, with great variations across different populations. The multisystem involvement of TTR amyloidosis is a clue to its diagnosis. For example, TTR amyloidosis diagnosis is considered when one or several of the following are present: (1) family history of neuropathic disease, especially associated with heart failure; (2) neuropathic pain or progressive sensory disturbances of unknown etiology; (3) carpal tunnel syndrome without obvious cause, particularly if it is bilateral and requires surgical release; (4) gastrointestinal motility disturbances or autonomic nerve dysfunction of unknown etiology (*e.g.,* erectile dysfunction,

orthostatic hypotension, neurogenic bladder); (5) cardiac disease characterized by thickened ventricular walls in the absence of hypertension; (6) advanced atrio-ventricular block of unknown origin, particularly when accompanied by a thickened heart; and (6) vitreous body inclusions of the cotton-wool type. See Ando *et al.*, *Orphanet Journal of Rare Diseases* 8:31 (2013). Other symptoms can include, for example, polyneuropathy, sensory loss, pain, weakness in lower limbs, dyhidrosis, diarrhea, constipation, weight loss, and urinary incontinence/retention.

**[00132]** Diagnosis of TTR amyloidosis typically relies on target organ biopsies, followed by histological staining of the excised tissue with the amyloid-specific dye, Congo red. If a positive test for amyloid is observed, immunohistochemical staining for TTR is subsequently performed to ensure that the precursor protein responsible for amyloid formation is indeed TTR. For familial forms of the diseases, demonstration of a mutation in the gene encoding TTR is then needed before diagnosis can be made. This can be accomplished, for example, through isoelectric focusing electrophoresis, polymerase chain reaction, or laser dissection/liquid chromatography-tandem mass spectrometry. See, *e.g.*, US 2014/0056904; Ruberg and Berk, *Circulation* 126:1286-1300 (2012); Ando *et al.*, *Orphanet Journal of Rare Diseases* 8:31 (2013).

#### **IV. Antibodies**

##### **A. Binding Specificity and Functional Properties**

**[00133]** The invention provides monoclonal antibodies binding to transthyretin (TTR) protein, more specifically, to epitopes within amino acid residues 89-97 (SEQ ID NO:35) of TTR. Such epitopes are buried in the native TTR tetramer and exposed in monomeric, misfolded, aggregated, or fibril forms of TTR.

**[00134]** An antibody designated 5A1 is such an exemplary mouse antibody. This antibody specifically binds within amino acid residues 89-97 (SEQ ID NO:35) of TTR. This antibody is further characterized by its ability to bind to monomeric, misfolded, aggregated, or fibril forms of TTR but not to native tetrameric forms of TTR. In addition, this antibody is characterized by its immunoreactivity on TTR-mediated amyloidosis cardiac tissue but not on healthy cardiac

tissue. Ability to bind to specific proteins or fragments thereof may be demonstrated using exemplary assay formats provided in the examples.

**[00135]** Some antibodies bind to the same or overlapping epitope as an antibody designated 5A1. The sequences of the heavy and light chain mature variable regions of 5A1 are designated SEQ ID NOS: 1 and 9, respectively. Other antibodies having such a binding specificity can be produced by immunizing mice with TTR, or a portion thereof including the desired epitope (*e.g.*, SEQ ID NO:35), and screening resulting antibodies for binding to monomeric TTR or a peptide comprising SEQ ID NO:35, optionally in competition with an antibody having the variable regions of mouse 5A1 (IgG1,kappa). Fragments of TTR including the desired epitope can be linked to a carrier that helps elicit an antibody response to the fragment and/or be combined with an adjuvant that helps elicit such a response. Such antibodies can be screened for differential binding to wild-type, monomeric versions of TTR or a fragment thereof (*e.g.*, SEQ ID NO:31) compared with mutants of specified residues. Screening against such mutants more precisely defines the binding specificity to allow identification of antibodies whose binding is inhibited by mutagenesis of particular residues and which are likely to share the functional properties of other exemplified antibodies. The mutations can be systematic replacement substitution with alanine (or serine if an alanine is present already) one residue at a time, or more broadly spaced intervals, throughout the target or throughout a section thereof in which an epitope is known to reside. If the same set of mutations significantly reduces the binding of two antibodies, the two antibodies bind the same epitope.

**[00136]** Antibodies having the binding specificity of a selected murine antibody (*e.g.*, 5A1) can also be produced using a variant of the phage display method. *See* Winter, WO 92/20791. This method is particularly suitable for producing human antibodies. In this method, either the heavy or light chain variable region of the selected murine antibody is used as a starting material. If, for example, a light chain variable region is selected as the starting material, a phage library is constructed in which members display the same light chain variable region (*i.e.*, the murine starting material) and a different heavy chain variable region. The heavy chain variable regions can for example be obtained from a library of rearranged human heavy chain variable regions. A phage showing strong specific binding (*e.g.*, at least  $10^8$  and preferably at least  $10^9$  M<sup>-1</sup>) for monomeric TTR or a fragment thereof (*e.g.*, amino acid residues 89-97) is selected. The heavy

chain variable region from this phage then serves as a starting material for constructing a further phage library. In this library, each phage displays the same heavy chain variable region (*i.e.*, the region identified from the first display library) and a different light chain variable region. The light chain variable regions can be obtained for example from a library of rearranged human variable light chain regions. Again, phage showing strong specific binding for monomeric TTR or a fragment thereof (*e.g.*, amino acid residues 89-97) are selected. The resulting antibodies usually have the same or similar epitope specificity as the murine starting material.

**[00137]** Other antibodies can be obtained by mutagenesis of cDNA encoding the heavy and light chains of an exemplary antibody, such as 5A1. Monoclonal antibodies that are at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identical to 5A1 in amino acid sequence of the mature heavy and/or light chain variable regions and maintain its functional properties, and/or which differ from the respective antibody by a small number of functionally inconsequential amino acid substitutions (*e.g.*, conservative substitutions), deletions, or insertions are also included in the invention. Monoclonal antibodies having at least one or all six CDR(s) as defined by conventional definition, but preferably Kabat, that are 90%, 95%, 99% or 100% identical to corresponding CDRs of 5A1 are also included.

**[00138]** The invention also provides antibodies having some or all (*e.g.*, 3, 4, 5, and 6) CDRs entirely or substantially from 5A1. Such antibodies can include a heavy chain variable region that has at least two, and usually all three, CDRs entirely or substantially from the heavy chain variable region of 5A1 and/or a light chain variable region having at least two, and usually all three, CDRs entirely or substantially from the light chain variable region of 5A1. The antibodies can include both heavy and light chains. A CDR is substantially from a corresponding 5A1 CDR when it contains no more than 4, 3, 2, or 1 substitutions, insertions, or deletions, except that CDRH2 (when defined by Kabat) can have no more than 6, 5, 4, 3, 2, or 1 substitutions, insertions, or deletions. Such antibodies can have at least 70%, 80%, 90%, 95%, 96%, 97%, 98%, or 99% identity to 5A1 in the amino acid sequence of the mature heavy and/or light chain variable regions and maintain their functional properties, and/or differ from 5A1 by a small number of functionally inconsequential amino acid substitutions (*e.g.*, conservative substitutions), deletions, or insertions.

**[00139]** Some antibodies identified by such assays can bind to monomeric, misfolded, aggregated, or fibril forms of TTR but not to native tetrameric forms of TTR, as described in the examples or otherwise. Likewise, some antibodies are immunoreactive on TTR-mediated amyloidosis tissue but not on healthy tissue.

**[00140]** Some antibodies can inhibit or reduce aggregation of TTR, inhibit or reduce TTR fibril formation, reduce or clear TTR deposits or aggregated TTR, or stabilize non-toxic conformations of TTR in an animal model or clinical trial. Some antibodies can treat, effect prophylaxis of, or delay the onset of a TTR amyloidosis as shown in an animal model or clinical trial. Exemplary animal models for testing activity against a TTR amyloidosis include those described in Kohno *et al.*, *Am. J. Path.* 150(4):1497-1508 (1997); Teng *et al.*, *Laboratory Investigations* 81:385-396 (2001); Wakasugi *et al.*, *Proc. Japan Acad.* 63B:344-347 (1987); Shimada *et al.*, *Mol. Biol. Med.* 6:333-343 (1989); Nagata *et al.*, *J. Biochem.* 117:169-175 (1995); Sousa *et al.*, *Am. J. Path.* 161:1935-1948 (2002); and Santos *et al.*, *Neurobiology of Aging* 31:280-289 (2010).

### **B. Non-Human Antibodies**

**[00141]** The production of other non-human antibodies, *e.g.*, murine, guinea pig, primate, rabbit or rat, against monomeric TTR or a fragment thereof (*e.g.*, amino acid residues 89-97) can be accomplished by, for example, immunizing the animal with TTR or a fragment thereof. *See* Harlow & Lane, *Antibodies, A Laboratory Manual* (CSHP NY, 1988) (incorporated by reference for all purposes). Such an immunogen can be obtained from a natural source, by peptide synthesis, or by recombinant expression. Optionally, the immunogen can be administered fused or otherwise complexed with a carrier protein. Optionally, the immunogen can be administered with an adjuvant. Several types of adjuvant can be used as described below. Complete Freund's adjuvant followed by incomplete adjuvant is preferred for immunization of laboratory animals. Rabbits or guinea pigs are typically used for making polyclonal antibodies. Mice are typically used for making monoclonal antibodies. Antibodies are screened for specific binding to monomeric TTR or an epitope within TTR (*e.g.*, an epitope comprising one or more of amino acid residues 89-97). Such screening can be accomplished by determining binding of an antibody to a collection of monomeric TTR variants, such as TTR variants containing amino acid



residues 89-97 or mutations within these residues, and determining which TTR variants bind to the antibody. Binding can be assessed, for example, by Western blot, FACS or ELISA.

### **C. *Humanized Antibodies***

**[00142]** A humanized antibody is a genetically engineered antibody in which CDRs from a non-human “donor” antibody are grafted into human “acceptor” antibody sequences (*see, e.g.*, Queen, US 5,530,101 and 5,585,089; Winter, US 5,225,539; Carter, US 6,407,213; Adair, US 5,859,205; and Foote, US 6,881,557). The acceptor antibody sequences can be, for example, a mature human antibody sequence, a composite of such sequences, a consensus sequence of human antibody sequences, or a germline region sequence. Thus, a humanized antibody is an antibody having at least three, four, five or all CDRs entirely or substantially from a donor antibody and variable region framework sequences and constant regions, if present, entirely or substantially from human antibody sequences. Similarly a humanized heavy chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody heavy chain, and a heavy chain variable region framework sequence and heavy chain constant region, if present, substantially from human heavy chain variable region framework and constant region sequences. Similarly a humanized light chain has at least one, two and usually all three CDRs entirely or substantially from a donor antibody light chain, and a light chain variable region framework sequence and light chain constant region, if present, substantially from human light chain variable region framework and constant region sequences. Other than nanobodies and dAbs, a humanized antibody comprises a humanized heavy chain and a humanized light chain. A CDR in a humanized antibody is substantially from a corresponding CDR in a non-human antibody when at least 85%, 90%, 95% or 100% of corresponding residues (as defined by any conventional definition but preferably defined by Kabat) are identical between the respective CDRs. The variable region framework sequences of an antibody chain or the constant region of an antibody chain are substantially from a human variable region framework sequence or human constant region respectively when at least 85%, 90%, 95% or 100% of corresponding residues defined by any conventional definition but preferably defined by Kabat are identical.

**[00143]** Although humanized antibodies often incorporate all six CDRs (preferably as defined by Kabat) from a mouse antibody, they can also be made with less than all CDRs (*e.g.*, at least 3,

4, or 5 CDRs) from a mouse antibody (*e.g.*, Pascalis *et al.*, *J. Immunol.* 169:3076, 2002; Vajdos *et al.*, *J. of Mol. Biol.*, 320: 415-428, 2002; Iwahashi *et al.*, *Mol. Immunol.* 36:1079-1091, 1999; Tamura *et al.*, *J. Immunol.*, 164:1432-1441, 2000).

**[00144]** In some antibodies only part of the CDRs, namely the subset of CDR residues required for binding, termed the SDRs, are needed to retain binding in a humanized antibody. CDR residues not contacting antigen and not in the SDRs can be identified based on previous studies (for example residues H60-H65 in CDR H2 are often not required), from regions of Kabat CDRs lying outside Chothia hypervariable loops (Chothia, *J. Mol. Biol.* 196:901, 1987), by molecular modeling and/or empirically, or as described in Gonzales *et al.*, *Mol. Immunol.* 41: 863, 2004. In such humanized antibodies at positions in which one or more donor CDR residues is absent or in which an entire donor CDR is omitted, the amino acid occupying the position can be an amino acid occupying the corresponding position (by Kabat numbering) in the acceptor antibody sequence. The number of such substitutions of acceptor for donor amino acids in the CDRs to include reflects a balance of competing considerations. Such substitutions are potentially advantageous in decreasing the number of mouse amino acids in a humanized antibody and consequently decreasing potential immunogenicity. However, substitutions can also cause changes of affinity, and significant reductions in affinity are preferably avoided. Positions for substitution within CDRs and amino acids to substitute can also be selected empirically.

**[00145]** The human acceptor antibody sequences can optionally be selected from among the many known human antibody sequences to provide a high degree of sequence identity (*e.g.*, 65-85% identity) between a human acceptor sequence variable region frameworks and corresponding variable region frameworks of a donor antibody chain.

**[00146]** Examples of acceptor sequences for the heavy chain are the human mature heavy chain variable region AGP01689 (SEQ ID NO:3) or other human heavy chain subgroup 1. The SEQ ID NO:3 acceptor sequence include two CDRs having the same canonical form as mouse 5A1 heavy chain. Examples of acceptor sequences for the light chain are the human mature light chain variable regions with NCBI accession code BAH04766 (SEQ ID NO:11) and other light chain variable regions of human kappa subgroup 2.

**[00147]** Certain amino acids from the human variable region framework residues can be selected for substitution based on their possible influence on CDR conformation and/or binding to antigen. Investigation of such possible influences is by modeling, examination of the characteristics of the amino acids at particular locations, or empirical observation of the effects of substitution or mutagenesis of particular amino acids.

**[00148]** For example, when an amino acid differs between a murine variable region framework residue and a selected human variable region framework residue, the human framework amino acid can be substituted by the equivalent framework amino acid from the mouse antibody when it is reasonably expected that the amino acid:

- (1) noncovalently binds antigen directly;
- (2) is adjacent to a CDR region or within a CDR as defined by Chothia but not Kabat;
- (3) otherwise interacts with a CDR region (*e.g.*, is within about 6 Å of a CDR region), (*e.g.*, identified by modeling the light or heavy chain on the solved structure of a homologous known immunoglobulin chain); or
- (4) is a residue participating in the VL-VH interface.

**[00149]** Framework residues from classes (1) through (3) as defined by Queen, US 5,530,101, are sometimes alternately referred to as canonical and vernier residues. Framework residues that help define the conformation of a CDR loop are sometimes referred to as canonical residues (Chothia & Lesk, *J. Mol. Biol.* 196:901-917 (1987); Thornton & Martin, *J. Mol. Biol.* 263:800-815 (1996)). Framework residues that support antigen-binding loop conformations and play a role in fine-tuning the fit of an antibody to antigen are sometimes referred to as vernier residues (Foote & Winter, *J. Mol. Biol.* 224:487-499 (1992)).

**[00150]** Other framework residues that are candidates for substitution are residues creating a potential glycosylation site. Still other candidates for substitution are acceptor human framework amino acids that are unusual for a human immunoglobulin at that position. These amino acids can be substituted with amino acids from the equivalent position of the mouse donor antibody or from the equivalent positions of more typical human immunoglobulins.

**[00151]** Exemplary humanized antibodies are humanized forms of the mouse 5A1 antibody. The mouse antibody comprises mature heavy and light chain variable regions having amino acid sequences comprising SEQ ID NO:1 and SEQ ID NO:9, respectively. The invention provides two exemplified humanized mature heavy chain variable regions: Hu5A1VHv1 and Hu5A1VHv2 (SEQ ID NOS:4 and 5). The invention further provides two exemplified human mature light chain variable regions: Hu5A1VLv1 and Hu5A1VLv2 (SEQ ID NOS:12 and 13).

**[00152]** Figures 1 and 2 show alignments of the heavy chain variable region and light chain variable region, respectively, of 5A1, mouse model antibodies, human acceptor antibodies, and humanized antibody versions of 5A1. The figures also show positions of CDRs, canonical residues, Vernier residues, and interface residues. Positions at which canonical, Vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. However, here the Figures show few such residues differing between mouse and human acceptor sequences. Heavy chain positions 29 and 93 were considered for substitution, because human and mouse residues differed at these positions. Position 29 is within the Chothia CDRH1 region and position 93 is a residue contributing to the VH-VL interface. Light chain positions 85 (within variable region framework) and 55 (within CDRL2) were considered for substitution on the basis that the residues occupying these position in a straight CDR graft were unusual for the positions in human antibodies. At position 85, the human acceptor T residue was replaced with the mouse V residue. At position 55, the mouse cysteine residue was replaced with a serine residue. Serine is not present in the human acceptor, so this substitution is neither a backmutation nor forward mutation. Three other variable region framework Vernier positions at which mouse and human acceptor residues differ are H49, H75 and L69. These positions can optionally backmutated (S49A, K75R, and T69A, respectively).

**[00153]** Here, as elsewhere, the first-mentioned residue is the residue of a humanized antibody formed by grafting Kabat CDRs or a composite Chothia Kabat CDR in the case of CDR-H1 into a human acceptor framework, and the second-mentioned residue is a residue being considered for replacing such residue. Thus, within variable region frameworks, the first mentioned residue is human (T85V, V29F, and A93H), and within CDRs, the first mentioned residue is mouse (C55S).

**[00154]** Exemplified antibodies include any permutations or combinations of the exemplified mature heavy and light chain variable regions (*e.g.*, VHv1/VLv1 or H1L1, VHv1/VLv2 or H1L2, VHv2/VLv1 or H2L1, and VHv2/VLv2 or H2L2). The invention provides variants of humanized antibodies in which the humanized mature heavy chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOS:4 or 5 and the humanized mature light chain variable region shows at least 90%, 95%, 96%, 97%, 98%, or 99% identity to SEQ ID NOS:12 or 13. In some such antibodies at least 1, 2, 3, or all 4 of the mutations present in any of SEQ ID NOS:4, 5, 12, or 13 are retained. In some antibodies, one or both of V<sub>H</sub> positions 29 and 93 is occupied by F and V respectively. In some antibodies, one or both of V<sub>L</sub> positions 55 and 85 is occupied by S and V respectively. The CDR regions of such humanized antibodies can be identical or substantially identical to the CDR regions the 5A1 mouse donor antibody. The CDR regions can be defined by any conventional definition (*e.g.*, Chothia, or composite of Chothia and Kabat) but are preferably as defined by Kabat.

**[00155]** Variable regions framework positions are in accordance with Kabat numbering unless otherwise stated. Other such variants typically differ from the sequences of the exemplified Hu5A1 heavy and light chains by a small number (*e.g.*, typically no more than 1, 2, 3, 5, 10, or 15) of replacements, deletions or insertions. Such differences are usually in the framework but can also occur in the CDRs.

**[00156]** A possibility for additional variation in humanized 5A1 variants is additional backmutations in the variable region frameworks. Many of the framework residues not in contact with the CDRs in the humanized mAb can accommodate substitutions of amino acids from the corresponding positions of the donor mouse mAb or other mouse or human antibodies, and even many potential CDR-contact residues are also amenable to substitution. Even amino acids within the CDRs may be altered, for example, with residues found at the corresponding position of the human acceptor sequence used to supply variable region frameworks. In addition, alternate human acceptor sequences can be used, for example, for the heavy and/or light chain. If different acceptor sequences are used, one or more of the backmutations recommended above may not be performed because the corresponding donor and acceptor residues are already the same without backmutations.

**[00157]** Preferably, replacements or backmutations in Hu5A1 variants (whether or not conservative) have no substantial effect on the binding affinity or potency of the humanized mAb, that is, its ability to bind to monomeric TTR (*e.g.*, the potency in some or all of the assays described in the present examples of the variant humanized 5A1 antibody is essentially the same, *i.e.*, within experimental error, as that of murine 5A1).

#### ***D. Chimeric and Veneered Antibodies***

**[00158]** The invention further provides chimeric and veneered forms of non-human antibodies, particularly the 5A1 antibodies of the examples.

**[00159]** A chimeric antibody is an antibody in which the mature variable regions of light and heavy chains of a non-human antibody (*e.g.*, a mouse) are combined with human light and heavy chain constant regions. Such antibodies substantially or entirely retain the binding specificity of the mouse antibody, and are about two-thirds human sequence.

**[00160]** A veneered antibody is a type of humanized antibody that retains some and usually all of the CDRs and some of the non-human variable region framework residues of a non-human antibody but replaces other variable region framework residues that may contribute to B- or T-cell epitopes, for example exposed residues (Padlan, *Mol. Immunol.* 28:489, 1991) with residues from the corresponding positions of a human antibody sequence. The result is an antibody in which the CDRs are entirely or substantially from a non-human antibody and the variable region frameworks of the non-human antibody are made more human-like by the substitutions. Veneered forms of the 5A1 antibody are included in the invention.

#### ***E. Human Antibodies***

**[00161]** Human antibodies against monomeric TTR or a fragment thereof (*e.g.*, amino acid residues 89-97 (SEQ ID NO:35) of TTR) are provided by a variety of techniques described below. Some human antibodies are selected by competitive binding experiments, by the phage display method of Winter, above, or otherwise, to have the same epitope specificity as a particular mouse antibody, such as one of the mouse monoclonal antibodies described in the examples. Human antibodies can also be screened for particular epitope specificity by using only a fragment of TTR, such as a TTR variant containing only amino acid residues 89-97 of

TTR, as the target antigen, and/or by screening antibodies against a collection of TTR variants, such as TTR variants containing various mutations within amino acid residues 89-97 of TTR.

**[00162]** Methods for producing human antibodies include the trioma method of Oestberg *et al.*, *Hybridoma* 2:361-367 (1983); Oestberg, U.S. Patent No. 4,634,664; and Engleman *et al.*, US Patent 4,634,666, use of transgenic mice including human immunoglobulin genes (*see, e.g.*, Lonberg *et al.*, WO93/12227 (1993); US 5,877,397; US 5,874,299; US 5,814,318; US 5,789,650; US 5,770,429; US 5,661,016; US 5,633,425; US 5,625,126; US 5,569,825; US 5,545,806; Neuberger, *Nat. Biotechnol.* 14:826 (1996); and Kucherlapati, WO 91/10741 (1991)) and phage display methods (*see, e.g.*, Dower *et al.*, WO 91/17271; McCafferty *et al.*, WO 92/01047; US 5,877,218; US 5,871,907; US 5,858,657; US 5,837,242; US 5,733,743; and US 5,565,332).

#### ***F. Selection of Constant Region***

**[00163]** The heavy and light chain variable regions of chimeric, veneered or humanized antibodies can be linked to at least a portion of a human constant region. The choice of constant region depends, in part, whether antibody-dependent cell-mediated cytotoxicity, antibody dependent cellular phagocytosis and/or complement dependent cytotoxicity are desired. For example, human isotopes IgG1 and IgG3 have complement-dependent cytotoxicity and human isotopes IgG2 and IgG4 do not. Human IgG1 and IgG3 also induce stronger cell mediated effector functions than human IgG2 and IgG4. Light chain constant regions can be lambda or kappa.

**[00164]** One or several amino acids at the amino or carboxy terminus of the light and/or heavy chain, such as the C-terminal lysine of the heavy chain, may be missing or derivatized in a proportion or all of the molecules. Substitutions can be made in the constant regions to reduce or increase effector function such as complement-mediated cytotoxicity or ADCC (*see, e.g.*, Winter *et al.*, US Patent No. 5,624,821; Tso *et al.*, US Patent No. 5,834,597; and Lazar *et al.*, *Proc. Natl. Acad. Sci. USA* 103:4005, 2006), or to prolong half-life in humans (*see, e.g.*, Hinton *et al.*, *J. Biol. Chem.* 279:6213, 2004). Exemplary substitutions include a Gln at position 250 and/or a Leu at position 428 (EU numbering is used in this paragraph for the constant region) for increasing the half-life of an antibody. Substitution at any or all of positions 234, 235, 236 and/or 237 reduce affinity for Fcγ receptors, particularly FcγRI receptor (*see, e.g.*, US

6,624,821). An alanine substitution at positions 234, 235, and 237 of human IgG1 can be used for reducing effector functions. Some antibodies have alanine substitution at positions 234, 235 and 237 of human IgG1 for reducing effector functions. Optionally, positions 234, 236 and/or 237 in human IgG2 are substituted with alanine and position 235 with glutamine (*see, e.g.*, US 5,624,821). In some antibodies, a mutation at one or more of positions 241, 264, 265, 270, 296, 297, 322, 329, and 331 by EU numbering of human IgG1 is used. In some antibodies, a mutation at one or more of positions 318, 320, and 322 by EU numbering of human IgG1 is used. In some antibodies, positions 234 and/or 235 are substituted with alanine and/or position 329 is substituted with glycine. In some antibodies, positions 234 and 235 are substituted with alanine, such as in SEQ ID NO:24. In some antibodies, the isotype is human IgG2 or IgG4.

**[00165]** An exemplary human light chain kappa constant region has the amino acid sequence of SEQ ID NO:25. The N-terminal arginine of SEQ ID NO:25 can be omitted, in which case light chain kappa constant region has the amino acid sequence of SEQ ID NO:26. An exemplary human IgG1 heavy chain constant region has the amino acid sequence of SEQ ID NO:22 (with or without the C-terminal lysine). Antibodies can be expressed as tetramers containing two light and two heavy chains, as separate heavy chains, light chains, as Fab, Fab', F(ab')<sub>2</sub>, and Fv, or as single chain antibodies in which heavy and light chain mature variable domains are linked through a spacer.

**[00166]** Human constant regions show allotypic variation and isoallotypic variation between different individuals, that is, the constant regions can differ in different individuals at one or more polymorphic positions. Isoallotypes differ from allotypes in that sera recognizing an isoallotype bind to a non-polymorphic region of a one or more other isotypes. Thus, for example, another heavy chain constant region is of IgG1 G1m3 allotype and has the amino acid sequence of SEQ ID NO:23. Another heavy chain constant region of the IgG1 G1m3 allotype has the amino acid sequence of SEQ ID NO:24 (with or without the C-terminal lysine). Reference to a human constant region includes a constant region with any natural allotype or any permutation of residues occupying positions in natural allotypes.



### **G. Expression of Recombinant Antibodies**

**[00167]** A number of methods are known for producing chimeric and humanized antibodies using an antibody-expressing cell line (*e.g.*, hybridoma). For example, the immunoglobulin variable regions of antibodies can be cloned and sequenced using well known methods. In one method, the heavy chain variable VH region is cloned by RT-PCR using mRNA prepared from hybridoma cells. Consensus primers are employed to the VH region leader peptide encompassing the translation initiation codon as the 5' primer and a g2b constant regions specific 3' primer. Exemplary primers are described in U.S. patent publication US 2005/0009150 by Schenk *et al.* (hereinafter "Schenk"). The sequences from multiple, independently derived clones can be compared to ensure no changes are introduced during amplification. The sequence of the VH region can also be determined or confirmed by sequencing a VH fragment obtained by 5' RACE RT-PCR methodology and the 3' g2b specific primer.

**[00168]** The light chain variable VL region can be cloned in an analogous manner. In one approach, a consensus primer set is designed for amplification of VL regions using a 5' primer designed to hybridize to the VL region encompassing the translation initiation codon and a 3' primer specific for the Ck region downstream of the V-J joining region. In a second approach, 5'RACE RT-PCR methodology is employed to clone a VL encoding cDNA. Exemplary primers are described in Schenk, *supra*. The cloned sequences are then combined with sequences encoding human (or other non-human species) constant regions. Exemplary sequences encoding human constant regions include SEQ ID NO:39, which encodes a human IgG1 constant region, and SEQ ID NOs:40 and 41, which encode a human kappa light chain constant region.

**[00169]** In one approach, the heavy and light chain variable regions are re-engineered to encode splice donor sequences downstream of the respective VDJ or VJ junctions and are cloned into a mammalian expression vector, such as pCMV-hy1 for the heavy chain and pCMV-Mc1 for the light chain. These vectors encode human  $\gamma$ 1 and Ck constant regions as exonic fragments downstream of the inserted variable region cassette. Following sequence verification, the heavy chain and light chain expression vectors can be co-transfected into CHO cells to produce chimeric antibodies. Conditioned media is collected 48 hours post-transfection and assayed by

western blot analysis for antibody production or ELISA for antigen binding. The chimeric antibodies are humanized as described above.

**[00170]** Chimeric, veneered, humanized, and human antibodies are typically produced by recombinant expression. Recombinant polynucleotide constructs typically include an expression control sequence operably linked to the coding sequences of antibody chains, including naturally associated or heterologous expression control elements, such as a promoter. The expression control sequences can be promoter systems in vectors capable of transforming or transfecting eukaryotic or prokaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences and the collection and purification of the crossreacting antibodies.

**[00171]** These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin resistance or hygromycin resistance, to permit detection of those cells transformed with the desired DNA sequences.

**[00172]** *E. coli* is one prokaryotic host useful for expressing antibodies, particularly antibody fragments. Microbes, such as yeast, are also useful for expression. *Saccharomyces* is a yeast host with suitable vectors having expression control sequences, an origin of replication, termination sequences, and the like as desired. Typical promoters include 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization.

**[00173]** Mammalian cells can be used for expressing nucleotide segments encoding immunoglobulins or fragments thereof. *See Winnacker, From Genes to Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed, and include CHO cell lines, various COS cell lines, HeLa cells, HEK293 cells, L cells, and non-antibody-producing myelomas including Sp2/0 and NS0. The cells can be nonhuman. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer (Queen *et al.*, *Immunol. Rev.* 89:49 (1986)), and necessary processing information sites, such as ribosome

binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Expression control sequences can include promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. *See Co et al., J. Immunol.* 148:1149 (1992).

**[00174]** Alternatively, antibody coding sequences can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal (*see, e.g.*, U.S. Pat. No. 5,741,957; U.S. Pat. No. 5,304,489; and U.S. Pat. No. 5,849,992). Suitable transgenes include coding sequences for light and/or heavy chains operably linked with a promoter and enhancer from a mammary gland specific gene, such as casein or beta lactoglobulin.

**[00175]** The vectors containing the DNA segments of interest can be transferred into the host cell by methods depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment, electroporation, lipofection, biolistics, or viral-based transfection can be used for other cellular hosts. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

**[00176]** Having introduced vector(s) encoding antibody heavy and light chains into cell culture, cell pools can be screened for growth productivity and product quality in serum-free media. Top-producing cell pools can then be subjected of FACS-based single-cell cloning to generate monoclonal lines. Specific productivities above 50 pg or 100 pg per cell per day, which correspond to product titers of greater than 7.5 g/L culture, can be used. Antibodies produced by single cell clones can also be tested for turbidity, filtration properties, PAGE, IEF, UV scan, HP-SEC, carbohydrate-oligosaccharide mapping, mass spectrometry, and binding assay, such as ELISA or Biacore. A selected clone can then be banked in multiple vials and stored frozen for subsequent use.

**[00177]** Once expressed, antibodies can be purified according to standard procedures of the art, including protein A capture, HPLC purification, column chromatography, gel electrophoresis and the like (*see generally*, Scopes, *Protein Purification* (Springer-Verlag, NY, 1982)).

**[00178]** Methodology for commercial production of antibodies can be employed, including codon optimization, selection of promoters, selection of transcription elements, selection of terminators, serum-free single cell cloning, cell banking, use of selection markers for amplification of copy number, CHO terminator, or improvement of protein titers (*see, e.g.*, US 5,786,464; US 6,114,148; US 6,063,598; US 7,569,339; W02004/050884; W02008/012142; W02008/012142; W02005/019442; W02008/107388; W02009/027471; and US 5,888,809).

#### **H. Antibody Screening Assays**

**[00179]** Antibodies can be subject to several screens including binding assays, functional screens, screens in animal models of diseases associated with TTR deposits, and clinical trials. Binding assays test for specific binding and, optionally, affinity and epitope specificity to monomeric TTR or a fragment thereof. For example, binding assays can screen for antibodies that bind to amino acid residues 89-97 (SEQ ID NO:35) of TTR, which is an epitope that is buried in the native TTR tetramer and exposed in monomeric, misfolded, aggregated, or fibril forms of TTR. Antibodies can also be screened for the ability to bind pre-fibrillar, non-native conformations of TTR and TTR amyloid fibrils but not native TTR conformations. For example, antibodies can be screened for the ability to bind to monomeric forms of TTR created by dissociation or disaggregation of native tetrameric TTR, and can be counter-screened against native tetrameric TTR, as described in the examples or otherwise. Likewise, antibodies can also be screened for their immunoreactivity on TTR-mediated amyloidosis tissue but not on healthy tissue. Such screens are sometimes performed in competition with an exemplary antibody, such as an antibody having the variable regions of 5A1 or IgG1 kappa isotype. Optionally, either the antibody or TTR target is immobilized in such assay.

**[00180]** Functional assays can be performed in cellular models including cells naturally expressing TTR or transfected with DNA encoding TTR or a fragment thereof. Suitable cells include cells derived from cardiac tissue or other tissues affected by TTR amyloidogenesis. Cells can be screened for reduced levels of monomeric, misfolded, aggregated, or fibril forms of

TTR (*e.g.*, by Western blotting or immunoprecipitation of cell extracts or supernatants) or reduced toxicity attributable to monomeric, misfolded, aggregated, or fibril forms of TTR. For example, antibodies can be tested for the ability to inhibit or reduce aggregation of TTR, inhibit or reduce TTR fibril formation, reduce TTR deposits, clear aggregated TTR, or stabilize non-toxic conformations of TTR.

**[00181]** Other functional assays can be performed in solution, such as testing whether an antibody is capable of disrupting or reducing TTR fibril formation when monomeric TTR or misfolded TTR intermediates in solution are contacted with the antibody. The extent of fibril formation can be probed by turbidity measurements, for example, at 400 nm on a UV-visible spectrometer equipped with a temperature control unit. Thioflavin-T can also be used to assess the extent of amyloid fibril formation. For example, a five-fold molar excess of Thioflavin-T can be added to TTR samples and left at room temperature for 30 minutes before measurements are taken. Thioflavin-T fluorescence can be monitored using a spectrofluorimeter. *See* US 2014/0056904.

**[00182]** Animal model screens test the ability of the antibody to therapeutically or prophylactically treat signs or symptoms in an animal model simulating a human disease associated with accumulation of TTR or TTR deposits. Such diseases include types of TTR amyloidosis, such as senile systemic amyloidosis (SSA), senile cardiac amyloidosis (SCA), familial amyloid polyneuropathy (FAP), familial amyloid cardiomyopathy (FAC), and central nervous system selective amyloidosis (CNSA). Suitable signs or symptoms that can be monitored include the presence and extent of amyloid deposits in various tissues, such as the gastrointestinal tract or heart. The extent of reduction of amyloid deposits can be determined by comparison with an appropriate control, such as the level of TTR amyloid deposits in control animals that have received a control antibody (*e.g.*, an isotype matched control antibody), a placebo, or no treatment at all. An exemplary animal model for testing activity against a TTR amyloidosis is a mouse model carrying a null mutation at the endogenous mouse *Ttr* locus and the human mutant *TTR* gene comprising a V30M mutation that is associated with familial amyloidotic polyneuropathy. *See, e.g.*, Kohno *et al.*, *Am. J. Path.* 150(4):1497-1508 (1997); Cardoso and Saraiva, *FASEB J* 20(2):234-239 (2006). Similar models also exist, including other models for familial versions of TTR amyloidosis and models for sporadic versions of TTR

amyloidosis. *See, e.g.,* Teng *et al.*, *Lab. Invest.* 81(3): 385-396 (2001); Ito and Maeda, *Mouse Models of Transthyretin Amyloidosis, in Recent Advances in Transthyretin Evolution, Structure, and Biological Functions*, pp. 261-280 (2009) (Springer Berlin Heidelberg). Transgenic animals can include a human TTR transgene, such as a TTR transgene with a mutation associated with TTR amyloidosis or a wild-type TTR transgene. To facilitate testing in animal models, chimeric antibodies having a constant region appropriate for the animal model can be used (*e.g.*, mouse-rat chimeras could be used for testing antibodies in rats). It can be concluded that a humanized version of an antibody will be effective if the corresponding mouse antibody or chimeric antibody is effective in an appropriate animal model and the humanized antibody has similar binding affinity (*e.g.*, within experimental error, such as by a factor of 1.5, 2, or 3).

**[00183]** Clinical trials test for safety and efficacy in a human having a disease associated with TTR amyloidosis.

#### ***I. Nucleic Acids***

**[00184]** The invention further provides nucleic acids encoding any of the heavy and light chains described above (*e.g.*, SEQ ID NOS:4, 5, 12, and 13). Optionally, such nucleic acids further encode a signal peptide and can be expressed with the signal peptide linked to the constant region (*e.g.*, signal peptides having amino acid sequences of SEQ ID NOS:42 (heavy chain) and 44 (light chain) that can be encoded by SEQ ID NOS:43, respectively (heavy chain) and 45, respectively (light chain)). Coding sequences of nucleic acids can be operably linked with regulatory sequences to ensure expression of the coding sequences, such as a promoter, enhancer, ribosome binding site, transcription termination signal, and the like. The nucleic acids encoding heavy and light chains can occur in isolated form or can be cloned into one or more vectors. The nucleic acids can be synthesized by, for example, solid state synthesis or PCR of overlapping oligonucleotides. Nucleic acids encoding heavy and light chains can be joined as one contiguous nucleic acid, *e.g.*, within an expression vector, or can be separate, *e.g.*, each cloned into its own expression vector.

### **J.      *Conjugated Antibodies***

**[00185]**    Conjugated antibodies that specifically bind to antigens exposed in pathogenic forms of TTR but not in native tetrameric forms of TTR, such as amino acid residues 89-97 (SEQ ID NO:35) of TTR, are useful in detecting the presence of monomeric, misfolded, aggregated, or fibril forms of TTR; monitoring and evaluating the efficacy of therapeutic agents being used to treat patients diagnosed with a TTR amyloidosis; inhibiting or reducing aggregation of TTR; inhibiting or reducing TTR fibril formation; reducing or clearing TTR deposits; stabilizing non-toxic conformations of TTR; or treating or effecting prophylaxis of a TTR amyloidosis in a patient. For example, such antibodies can be conjugated with other therapeutic moieties, other proteins, other antibodies, and/or detectable labels. *See* WO 03/057838; US 8,455,622.

**[00186]**    Conjugated therapeutic moieties can be any agent that can be used to treat, combat, ameliorate, prevent, or improve an unwanted condition or disease in a patient, such as a TTR amyloidosis. Therapeutic moieties can include, for example, immunomodulators or any biologically active agents that facilitate or enhance the activity of the antibody. An immunomodulator can be any agent that stimulates or inhibits the development or maintenance of an immunologic response. If such therapeutic moieties are coupled to an antibody specific for monomeric, misfolded, aggregated, or fibril forms of TTR, such as the antibodies described herein, the coupled therapeutic moieties will have a specific affinity for non-native, pathogenic forms of TTR over native tetrameric forms of TTR. Consequently, administration of the conjugated antibodies directly targets tissues comprising pathogenic forms of TTR with minimal damage to surrounding normal, healthy tissue. This can be particularly useful for therapeutic moieties that are too toxic to be administered on their own. In addition, smaller quantities of the therapeutic moieties can be used.

**[00187]**    Examples of suitable therapeutic moieties include drugs that reduce levels of TTR, stabilize the native tetrameric structure of TTR, inhibit aggregation of TTR, disrupt TTR fibril or amyloid formation, or counteract cellular toxicity. *See, e.g.,* Almeida and Saraiva, *FEBS Letters* 586:2891-2896 (2012); Saraiva, *FEBS Letters* 498:201-203 (2001); Ando *et al.*, *Orphanet Journal of Rare Diseases* 8:31 (2013); Ruberg and Berk, *Circulation* 126:1286-1300 (2012); and Johnson *et al.*, *J. Mol. Biol.* 421(2-3):185-203 (2012). For example, antibodies can be

conjugated to tafamidis, diflunisal, ALN-TTR01, ALNTTR02, ISIS-TTRRx, doxycycline (doxy), tauroursodeoxycholic acid (TUDCA), Doxy-TUDCA, epigallocatechin gallate (EGCG), curcumin, or resveratrol (3,5,4'-trihydroxystilbene). Other representative therapeutic moieties include other agents known to be useful for treatment, management, or amelioration of a TTR amyloidosis or symptoms of a TTR amyloidosis. *See, e.g., Ando et al., Orphanet Journal of Rare Diseases* 8:31 (2013) for common clinical symptoms of TTR amyloidosis and typical agents used to treat those symptoms.

**[00188]** Antibodies can also be coupled with other proteins. For example, antibodies can be coupled with Fynomers. Fynomers are small binding proteins (*e.g.*, 7 kDa) derived from the human Fyn SH3 domain. They can be stable and soluble, and they can lack cysteine residues and disulfide bonds. Fynomers can be engineered to bind to target molecules with the same affinity and specificity as antibodies. They are suitable for creating multi-specific fusion proteins based on antibodies. For example, Fynomers can be fused to N-terminal and/or C-terminal ends of antibodies to create bi- and tri-specific FynomAbs with different architectures. Fynomers can be selected using Fynomer libraries through screening technologies using FACS, Biacore, and cell-based assays that allow efficient selection of Fynomers with optimal properties. Examples of Fynomers are disclosed in Grabulovski *et al.*, *J. Biol. Chem.* 282:3196-3204 (2007); Bertschinger *et al.*, *Protein Eng. Des. Sel.* 20:57-68 (2007); Schlatter *et al.*, *MAbs.* 4:497-508 (2011); Banner *et al.*, *Acta. Crystallogr. D. Biol. Crystallogr.* 69(Pt6):1124-1137 (2013); and Brack *et al.*, *Mol. Cancer Ther.* 13:2030-2039 (2014).

**[00189]** The antibodies disclosed herein can also be coupled or conjugated to one or more other antibodies (*e.g.*, to form antibody heteroconjugates). Such other antibodies can bind to different epitopes within TTR or a portion thereof or can bind to a different target antigen.

**[00190]** Antibodies can also be coupled with a detectable label. Such antibodies can be used, for example, for diagnosing a TTR amyloidosis, for monitoring progression of a TTR amyloidosis, and/or for assessing efficacy of treatment. Such antibodies are particularly useful for performing such determinations in subjects having or being susceptible to a TTR amyloidosis, or in appropriate biological samples obtained from such subjects. Representative detectable labels that may be coupled or linked to a humanized 5A1 antibody include various



enzymes, such as horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such streptavidin/biotin and avidin/biotin; fluorescent materials, such as umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as luminol; bioluminescent materials, such as luciferase, luciferin, and aequorin; radioactive materials, such as yttrium<sup>90</sup> (90Y), radiosilver-111, radiosilver-199, Bismuth<sup>213</sup>, iodine (<sup>131</sup>I, <sup>125</sup>I, <sup>123</sup>I, <sup>121</sup>I), carbon (<sup>14</sup>C), sulfur (<sup>35</sup>S), tritium (<sup>3</sup>H), indium (<sup>115</sup>In, <sup>113</sup>In, <sup>112</sup>In, <sup>111</sup>In), technetium (<sup>99</sup>Tc), thallium (<sup>201</sup>Tl), gallium (<sup>68</sup>Ga, <sup>67</sup>Ga), palladium (<sup>103</sup>Pd), molybdenum (<sup>99</sup>Mo), xenon (<sup>133</sup>Xe), fluorine (<sup>18</sup>F), <sup>153</sup>Sm, <sup>177</sup>Lu, <sup>159</sup>Gd, <sup>149</sup>Pm, <sup>140</sup>La, <sup>175</sup>Yb, <sup>166</sup>Ho, <sup>90</sup>Y, <sup>47</sup>Sc, <sup>186</sup>Re, <sup>188</sup>Re, <sup>142</sup>Pr, <sup>105</sup>Rh, <sup>97</sup>Ru, <sup>68</sup>Ge, <sup>57</sup>Co, <sup>65</sup>Zn, <sup>85</sup>Sr, <sup>32</sup>P, <sup>153</sup>Gd, <sup>169</sup>Yb, <sup>51</sup>Cr, <sup>54</sup>Mn, <sup>75</sup>Se, <sup>113</sup>Sn, and <sup>117</sup>Tm; positron emitting metals using various positron emission tomographies; nonradioactive paramagnetic metal ions; and molecules that are radiolabelled or conjugated to specific radioisotopes.

**[00191]** Linkage of radioisotopes to antibodies may be performed with conventional bifunction chelates. For radiosilver-111 and radiosilver-199 linkage, sulfur-based linkers may be used. *See Hazra et al., Cell Biophys.* 24-25:1-7 (1994). Linkage of silver radioisotopes may involve reducing the immunoglobulin with ascorbic acid. For radioisotopes such as 111In and 90Y, ibritumomab tiuxetan can be used and will react with such isotopes to form 111In-ibritumomab tiuxetan and 90Y-ibritumomab tiuxetan, respectively. *See Witzig, Cancer Chemother. Pharmacol.*, 48 Suppl 1:S91-S95 (2001).

**[00192]** Therapeutic moieties, other proteins, other antibodies, and/or detectable labels may be coupled or conjugated, directly or indirectly through an intermediate (*e.g.*, a linker), to a murine, chimeric, veneered, or humanized 5A1 antibody 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.*, 62:119-58 (1982). Suitable linkers include, for example, cleavable and non-cleavable linkers. Different linkers that release the coupled therapeutic moieties, proteins, antibodies, and/or detectable labels under acidic or reducing conditions, on exposure to specific proteases, or under other defined conditions can be employed.

## V. Therapeutic Applications

**[00193]** The above antibodies can be used for treating or effecting prophylaxis of a disease in a patient having or at risk for the disease mediated at least in part by transthyretin (TTR), and particularly by monomeric, misfolded, aggregated, or fibril forms of TTR. Although an understanding of mechanism is not required for practice, it is believed that any or all of the following mechanisms may contribute to treatment of TTR amyloidosis using the above antibodies: antibody-mediated inhibition of TTR aggregation and fibril formation, antibody-mediated stabilization of non-toxic conformations of TTR (*e.g.*, tetrameric forms), or antibody-mediated clearance of aggregated TTR, oligomeric TTR, or monomeric TTR. Antibody-drug conjugates can have additional mechanisms of action determined by the conjugated moiety.

**[00194]** Antibodies are administered in an effective regime meaning a dosage, route of administration and frequency of administration that delays the onset, reduces the severity, inhibits further deterioration, and/or ameliorates at least one sign or symptom of a disorder being treated. If a patient is already suffering from a disorder, the regime can be referred to as a therapeutically effective regime. If the patient is at elevated risk of the disorder relative to the general population but is not yet experiencing symptoms, the regime can be referred to as a prophylactically effective regime. In some instances, therapeutic or prophylactic efficacy can be observed in an individual patient relative to historical controls or past experience in the same patient. In other instances, therapeutic or prophylactic efficacy can be demonstrated in a preclinical or clinical trial in a population of treated patients relative to a control population of untreated patients.

**[00195]** The frequency of administration depends on the half-life of the antibody in the circulation, the condition of the patient and the route of administration among other factors. The

frequency can be daily, weekly, monthly, quarterly, or at irregular intervals in response to changes in the patient's condition or progression of the disorder being treated. An exemplary frequency for intravenous administration is between weekly and quarterly over a continuous course of treatment, although more or less frequent dosing is also possible. For subcutaneous administration, an exemplary dosing frequency is daily to monthly, although more or less frequent dosing is also possible.

**[00196]** The number of dosages administered depends on whether the disorder is acute or chronic and the response of the disorder to the treatment. For acute disorders or acute exacerbations of a chronic disorder, between 1 and 10 doses are often sufficient. Sometimes a single bolus dose, optionally in divided form, is sufficient for an acute disorder or acute exacerbation of a chronic disorder. Treatment can be repeated for recurrence of an acute disorder or acute exacerbation. For chronic disorders, an antibody can be administered at regular intervals, *e.g.*, weekly, fortnightly, monthly, quarterly, every six months for at least 1, 5 or 10 years, or the life of the patient.

## **VI. Pharmaceutical Compositions and Methods of Use**

**[00197]** Provided herein are several methods of diagnosing, monitoring, treating or effecting prophylaxis of diseases or conditions mediated at least in part by transthyretin (TTR), and particularly by monomeric, misfolded, aggregated, or fibril forms of TTR (*e.g.*, TTR amyloidosis). Examples of such diseases include familial TTR amyloidoses, such as familial amyloid cardiomyopathy (FAC), familial amyloid polyneuropathy (FAP), or central nervous system selective amyloidosis (CNSA), and sporadic TTR amyloidoses, such as senile systemic amyloidosis (SSA) or senile cardiac amyloidosis (SCA). Antibodies described above can be incorporated into a pharmaceutical composition for use in such methods. In general, an antibody or pharmaceutical composition containing an antibody is administered to a subject in need thereof. Patients amenable to treatment include individuals at risk of TTR amyloidosis but not showing symptoms, as well as patients presently showing symptoms. Some patients can be treated during the prodromal stage of TTR amyloidosis.

**[00198]** The pharmaceutical compositions can be administered prophylactically to individuals who have a known genetic risk of TTR amyloidosis. Such individuals include those having

relatives who have experienced such a disease, and those whose risk is determined by analysis of genetic or biochemical markers (*e.g.*, mutations in TTR associated with TTR amyloidosis), including using the diagnostic methods provided herein. For example, there are over 100 mutations in the gene encoding TTR that have been implicated in TTR amyloidosis. *See, e.g.*, US 2014/0056904; Saraiva, *Hum. Mutat.* 17(6):493-503 (2001); Damas and Saraiva, *J. Struct. Biol.* 130:290-299; Dwulet and Benson, *Biochem. Biophys. Res. Commun.* 114:657-662 (1983).

**[00199]** Individuals suffering from TTR amyloidosis can sometimes be recognized from the clinical manifestations of TTR amyloidosis, including one or more of the following: (1) family history of neuropathic disease, especially associated with heart failure; (2) neuropathic pain or progressive sensory disturbances of unknown etiology; (3) carpal tunnel syndrome without obvious cause, particularly if it is bilateral and requires surgical release; (4) gastrointestinal motility disturbances or autonomic nerve dysfunction of unknown etiology (*e.g.*, erectile dysfunction, orthostatic hypotension, neurogenic bladder); (5) cardiac disease characterized by thickened ventricular walls in the absence of hypertension; (6) advanced atrio-ventricular block of unknown origin, particularly when accompanied by a thickened heart; and (6) vitreous body inclusions of the cotton-wool type. *See Ando et al., Orphanet Journal of Rare Diseases* 8:31 (2013). Definitive diagnosis of TTR amyloidosis, however, typically relies on target organ biopsies, followed by histological staining of the excised tissue with the amyloid-specific dye, Congo red. If a positive test for amyloid is observed, immunohistochemical staining for TTR is subsequently performed to ensure that the precursor protein responsible for amyloid formation is indeed TTR. For familial forms of the diseases, demonstration of a mutation in the gene encoding TTR is then needed before a definitive diagnosis can be made.

**[00200]** The identification of the subject can occur in a clinical setting, or elsewhere, such as in the subject's home, for example, through the subject's own use of a self-testing kit. For example, the subject can be identified based on various symptoms such as peripheral neuropathy (sensory and motor), autonomic neuropathy, gastrointestinal impairment, cardiomyopathy, nephropathy, or ocular deposition. *See Ando et al., Orphanet Journal of Rare Diseases* 8:31 (2013). The subject can also be identified by increased levels of non-native forms of TTR in plasma samples from the subject compared to control samples, as disclosed in the examples.

**[00201]** As warranted by family history, genetic testing, or medical screening for TTR amyloidosis, treatment can begin at any age (*e.g.*, 20, 30, 40, 50, 60, or 70 years of age). Treatment typically entails multiple dosages over a period of time and can be monitored by assaying antibody or activated T-cell or B-cell responses to a therapeutic agent (*e.g.*, a truncated form of TTR comprising amino acid residues 89-97) over time. If the response falls, a booster dosage is indicated.

**[00202]** In prophylactic applications, an antibody or a pharmaceutical composition of the same is administered to a subject susceptible to, or otherwise at risk of a disease (*e.g.*, TTR amyloidosis) in a regime (dose, frequency and route of administration) effective to reduce the risk, lessen the severity, or delay the onset of at least one sign or symptom of the disease. In therapeutic applications, an antibody or immunogen to induce an antibody is administered to a subject suspected of, or already suffering from a disease (*e.g.*, TTR amyloidosis) in a regime (dose, frequency and route of administration) effective to ameliorate or at least inhibit further deterioration of at least one sign or symptom of the disease.

**[00203]** A regime is considered therapeutically or prophylactically effective if an individual treated subject achieves an outcome more favorable than the mean outcome in a control population of comparable subjects not treated by methods disclosed herein, or if a more favorable outcome is demonstrated for a regime in treated subjects versus control subjects in a controlled clinical trial (*e.g.*, a phase II, phase II/III, or phase III trial) or an animal model at the  $p < 0.05$  or 0.01 or even 0.001 level.

**[00204]** An effective regime of an antibody can be used for, *e.g.*, inhibiting or reducing aggregation of TTR in a subject having or at risk of a condition associated with TTR accumulation; inhibiting or reducing TTR fibril formation in a subject having or at risk of a condition associated with TTR accumulation; reducing or clearing TTR deposits or aggregated TTR in a subject having or at risk of a condition associated with TTR accumulation; stabilizing non-toxic conformations of TTR in a subject having or at risk of a condition associated with TTR accumulation; inhibiting toxic effects of TTR aggregates, fibrils or deposits in a subject having or at risk of a condition associated with TTR accumulation; diagnosing the presence or absence of TTR amyloid accumulation in a tissue suspected of comprising the amyloid accumulation;

determining a level of TTR deposits in a subject by detecting the presence of bound antibody in the subject following administration of the antibody; detecting the presence of monomeric, misfolded, aggregated, or fibril forms of TTR in a subject; monitoring and evaluating the efficacy of therapeutic agents being used to treat patients diagnosed with a TTR amyloidosis; inducing an immune response comprising antibodies to TTR in a subject; delaying the onset of a condition associated with TTR amyloid accumulation in a subject; or treating or effecting prophylaxis of a TTR amyloidosis in a patient.

**[00205]** Effective doses vary depending on many different factors, such as means of administration, target site, physiological state of the subject, whether the subject is human or an animal, other medications administered, and whether treatment is prophylactic or therapeutic.

**[00206]** An exemplary dose range for antibodies can be from about 0.1-20, or 0.5-5 mg/kg body weight (*e.g.*, 0.5, 1, 2, 3, 4 or 5 mg/kg) or 10-1500 mg as a fixed dosage. The dosage depends on the condition of the patient and response to prior treatment, if any, whether the treatment is prophylactic or therapeutic and whether the disorder is acute or chronic, among other factors.

**[00207]** Antibody can be administered in such doses daily, on alternative days, weekly, fortnightly, monthly, quarterly, or according to any other schedule determined by empirical analysis. An exemplary treatment entails administration in multiple doses over a prolonged period, for example, of at least six months. Additional exemplary treatment regimes entail administration once per every two weeks or once a month or once every 3 to 6 months.

**[00208]** Antibodies can be administered via a peripheral route. Routes of administration include topical, intravenous, oral, subcutaneous, intraarterial, intracranial, intrathecal, intraperitoneal, intranasal or intramuscular. Routes for administration of antibodies can be intravenous or subcutaneous. Intravenous administration can be, for example, by infusion over a period such as 30-90 min. This type of injection is most typically performed in the arm or leg muscles. In some methods, agents are injected directly into a particular tissue where deposits have accumulated, for example intracranial injection.

**[00209]** Pharmaceutical compositions for parenteral administration can be sterile and substantially isotonic (250-350 mOsm/kg water) and manufactured under GMP conditions. Pharmaceutical compositions can be provided in unit dose form (*i.e.*, the dose for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries. The formulation depends on the route of administration chosen. For injection, antibodies can be formulated in aqueous solutions, *e.g.*, in physiologically compatible buffers such as Hank's solution, Ringer's solution, or physiological saline or acetate buffer (to reduce discomfort at the site of injection). The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively antibodies can be in lyophilized form for constitution with a suitable vehicle, *e.g.*, sterile pyrogen-free water, before use.

**[00210]** The regimes can be administered in combination with another agent effective in treatment or prophylaxis of the disease being treated. Such agents can include siRNA to inhibit expression of TTR or Vyndaqel, a stabilizer of TTR in tetramer formation.

**[00211]** After treatment, the subject's condition can be evaluated to determine the progress or efficacy of such treatment. Such methods preferably test for changes in TTR amyloid levels or levels of non-native forms of TTR. For example, TTR amyloid levels may be evaluated to determine improvement relative to the subject's TTR amyloid levels under comparable circumstances prior to treatment. The subject's TTR amyloid levels can also be compared with control populations under comparable circumstances. The control populations can be similarly afflicted, untreated subjects or normal untreated subjects (among other control subjects). Improvement relative to similarly afflicted, untreated subjects or levels approaching or reaching the levels in untreated normal subjects indicates a positive response to treatment.

**[00212]** TTR amyloid levels can be measured by a number of methods, including imaging techniques. Examples of suitable imaging techniques include PET scanning with radiolabeled TTR or fragments thereof, TTR antibodies or fragments thereof, Congo-red-based amyloid imaging agents, such as, *e.g.*, PIB (US 2011/0008255), amyloid-imaging peptide p31 (Biodistribution of amyloid-imaging peptide, p31, correlates with amyloid quantitation based on Congo red tissue staining, Wall *et al.*, Abstract No. 1573, 2011 ISNM Annual Meeting), and

other PET labels. Levels of non-native forms of TTR can be measured, for example, by performing SDS-PAGE/Western blot or Meso Scale Discovery plate assays with the antibodies disclosed herein on plasma samples or biopsy samples from a subject and comparing to control samples, as described in the examples.

#### **A.     *Diagnostics and Monitoring Methods***

**[00213]** Also provided are methods of detecting an immune response against TTR in a patient suffering from or susceptible to diseases associated with TTR deposition or pathogenic forms of TTR (*e.g.*, monomeric, misfolded, aggregated, or fibril forms of TTR). The methods can be used to monitor a course of therapeutic and prophylactic treatment with the agents provided herein. The antibody profile following passive immunization typically shows an immediate peak in antibody concentration followed by an exponential decay. Without a further dose, the decay approaches pretreatment levels within a period of days to months depending on the half-life of the antibody administered. For example, the half-life of some human antibodies is of the order of 20 days.

**[00214]** In some methods, a baseline measurement of antibody to TTR in the subject is made before administration, a second measurement is made soon thereafter to determine the peak antibody level, and one or more further measurements are made at intervals to monitor decay of antibody levels. When the level of antibody has declined to baseline or a predetermined percentage of the peak less baseline (*e.g.*, 50%, 25% or 10%), administration of a further dose of antibody is administered. In some methods, peak or subsequent measured levels less background are compared with reference levels previously determined to constitute a beneficial prophylactic or therapeutic treatment regime in other subjects. If the measured antibody level is significantly less than a reference level (*e.g.*, less than the mean minus one or, preferably, two standard deviations of the reference value in a population of subjects benefiting from treatment) administration of an additional dose of antibody is indicated.

**[00215]** Also provided are methods of detecting monomeric, misfolded, aggregated, or fibril forms of TTR in a subject, for example, by measuring TTR amyloid or pathogenic forms of TTR (*e.g.*, monomeric, misfolded, aggregated, or fibril forms of TTR) in a sample from a subject or by *in vivo* imaging of TTR in a subject. Such methods are useful to diagnose or confirm



diagnosis of diseases associated with such pathogenic forms of TTR (*e.g.*, TTR amyloidosis), or susceptibility thereto. The methods can also be used on asymptomatic subjects. The presence of monomeric, misfolded, aggregated, or fibril forms of TTR indicates susceptibility to future symptomatic disease. The methods are also useful for monitoring disease progression and/or response to treatment in subjects who have been previously diagnosed with a TTR amyloidosis.

**[00216]** Biological samples obtained from a subject having, suspected of having, or at risk of having a TTR amyloidosis can be contacted with the antibodies disclosed herein to assess the presence of monomeric, misfolded, aggregated, or fibril forms of TTR. For example, levels of monomeric, misfolded, aggregated, or fibril forms of TTR in such subjects may be compared to those present in healthy subjects. Alternatively, levels of TTR amyloid or pathogenic forms of TTR (*e.g.*, monomeric, misfolded, aggregated, or fibril forms of TTR) in such subjects receiving treatment for the disease may be compared to those of subjects who have not been treated for a TTR amyloidosis. Some such tests involve a biopsy of tissue obtained from such subjects. ELISA assays may also be useful methods, for example, for assessing levels of monomeric, misfolded, aggregated, or fibril forms of TTR in fluid samples. Some such ELISA assays involve anti-TTR antibodies that preferentially bind monomeric, misfolded, aggregated, or fibril forms of TTR relative to normal tetrameric forms of TTR.

**[00217]** The *in vivo* imaging methods can work by administering a reagent, such as antibody that binds to monomeric, misfolded, aggregated, or fibril forms of TTR in the subject, and then detecting the reagent after it has bound. Such antibodies typically bind to an epitope within residues 89-97 of TTR. If desired, the clearing response can be avoided by using antibody fragments lacking a full length constant region, such as Fabs. In some methods, the same antibody can serve as both a treatment and diagnostic reagent.

**[00218]** Diagnostic reagents can be administered by intravenous injection into the body of the subject, or via other routes deemed reasonable. The dose of reagent should be within the same ranges as for treatment methods. Typically, the reagent is labeled, although in some methods, the primary reagent with affinity for monomeric, misfolded, aggregated, or fibril forms of TTR is unlabeled and a secondary labeling agent is used to bind to the primary reagent. The choice of label depends on the means of detection. For example, a fluorescent label is suitable for optical

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

**[00219]** Diagnosis is performed by comparing the number, size, and/or intensity of labeled loci to corresponding base line values. The base line values can represent the mean levels in a population of undiseased individuals. Base line values can also represent previous levels determined in the same subject. For example, base line values can be determined in a subject before beginning treatment, and measured values thereafter compared with the base line values. A decrease in values relative to base line generally signals a positive response to treatment.

## **IX. Kits**

**[00220]** The invention further provides kits (*e.g.*, containers) comprising the humanized 5A1 antibodies disclosed herein and related materials, such as instructions for use (*e.g.*, package insert). The instructions for use may contain, for example, instructions for administration of the antibodies and optionally one or more additional agents. The containers of antibodies may be unit doses, bulk packages (*e.g.*, multi-dose packages), or sub-unit doses.

**[00221]** Package insert refers to instructions customarily included in commercial packages of therapeutic products that contain information about the indications, usage, dosage, administration, contraindications and/or warnings concerning the use of such therapeutic products

**[00222]** Kits can also include a second container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It can also include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

## **X. Other Applications**

**[00223]** The antibodies can be used for detecting monomeric, misfolded, aggregated, or fibril forms of transthyretin (TTR), or fragments thereof, in the context of clinical diagnosis or treatment or in research. For example, the antibodies can be used to detect the presence of monomeric, misfolded, aggregated, or fibril forms of TTR in a biological sample as an indication

that the biological sample comprises TTR amyloid deposits. Binding of the antibodies to the biological sample can be compared to binding of the antibodies to a control sample. The control sample and the biological sample can comprise cells of the same tissue origin. Control samples and biological samples can be obtained from the same individual or different individuals and on the same occasion or on different occasions. If desired, multiple biological samples and multiple control samples are evaluated on multiple occasions to protect against random variation independent of the differences between the samples. A direct comparison can then be made between the biological sample(s) and the control sample(s) to determine whether antibody binding (*i.e.*, the presence of monomeric, misfolded, aggregated, or fibril forms of TTR) to the biological sample(s) is increased, decreased, or the same relative to antibody binding to the control sample(s). Increased binding of the antibody to the biological sample(s) relative to the control sample(s) indicates the presence of monomeric, misfolded, aggregated, or fibril forms of TTR in the biological sample(s). In some instances, the increased antibody binding is statistically significant. Optionally, antibody binding to the biological sample is at least 1.5-fold, 2-fold, 3-fold, 4-fold, 5-fold, 10-fold, 20-fold, or 100-fold higher than antibody binding to the control sample.

**[00224]** In addition, the antibodies can be used to detect the presence of monomeric, misfolded, aggregated, or fibril forms of TTR in a biological sample to monitor and evaluate the efficacy of a therapeutic agent being used to treat a patient diagnosed with a TTR amyloidosis. A biological sample from a patient diagnosed with a TTR amyloidosis is evaluated to establish a baseline for the binding of the antibodies to the sample (*i.e.*, a baseline for the presence of the monomeric, misfolded, aggregated, or fibril forms of TTR in the sample) before commencing therapy with the therapeutic agent. In some instances, multiple biological samples from the patient are evaluated on multiple occasions to establish both a baseline and measure of random variation independent of treatment. A therapeutic agent is then administered in a regime. The regime may include multiple administrations of the agent over a period of time. Optionally, binding of the antibodies (*i.e.*, presence of monomeric, misfolded, aggregated, or fibril forms of TTR) is evaluated on multiple occasions in multiple biological samples from the patient, both to establish a measure of random variation and to show a trend in response to immunotherapy. The various assessments of antibody binding to the biological samples are then compared. If only two assessments are made, a direct comparison can be made between the two assessments to

determine whether antibody binding (*i.e.*, presence of monomeric, misfolded, aggregated, or fibril forms of TTR) has increased, decreased, or remained the same between the two assessments. If more than two measurements are made, the measurements can be analyzed as a time course starting before treatment with the therapeutic agent and proceeding through the course of therapy. In patients for whom antibody binding to biological samples has decreased (*i.e.*, the presence of monomeric, misfolded, aggregated, or fibril forms of TTR), it can be concluded that the therapeutic agent was effective in treating the TTR amyloidosis in the patient. The decrease in antibody binding can be statistically significant. Optionally, binding decreases by at least 1%, 2%, 3%, 4%, 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, or 100%. Assessment of antibody binding can be made in conjunction with assessing other signs and symptoms of TTR amyloidosis.

**[00225]** The antibodies can also be used as research reagents for laboratory research in detecting monomeric, misfolded, aggregated, or fibril forms of TTR, or fragments thereof. In such uses, antibodies can be labeled with fluorescent molecules, spin-labeled molecules, enzymes, or radioisotopes, and can be provided in the form of kit with all the necessary reagents to perform the detection assay. The antibodies can also be used to purify monomeric, misfolded, aggregated, or fibril forms of TTR, or binding partners of monomeric, misfolded, aggregated, or fibril forms of TTR, *e.g.*, by affinity chromatography.

**[00226]** The antibodies can also be used for inhibiting or reducing aggregation of TTR, inhibiting or reducing TTR fibril formation, reducing or clearing TTR deposits or TTR aggregates, or stabilizing non-toxic conformations of TTR in a biological sample. The biological sample can comprise, for example, blood, serum, plasma, or tissue (*e.g.*, tissue from the heart, peripheral nervous system, autonomic nervous system, kidneys, eyes, or gastrointestinal tract). In some instances, TTR aggregation, TTR fibril formation, or TTR deposits are inhibited or reduced by at least 10%, 20%, 25%, 30%, 40%, 50%, or 75%, (*e.g.*, 10%-75% or 30%-70%). Assays for detecting fibril formation are described elsewhere herein. *See also* US 2014/0056904.

**[00227]** All patent filings, websites, other publications, accession numbers and the like cited above or below are incorporated by reference in their entirety for all purposes to the same extent

as if each individual item were specifically and individually indicated to be so incorporated by reference. If different versions of a sequence are associated with an accession number at different times, the version associated with the accession number at the effective filing date of this application is meant. The effective filing date means the earlier of the actual filing date or filing date of a priority application referring to the accession number if applicable. Likewise if different versions of a publication, website or the like are published at different times, the version most recently published at the effective filing date of the application is meant unless otherwise indicated. Any feature, step, element, embodiment, or aspect of the invention can be used in combination with any other unless specifically indicated otherwise. Although the present invention has been described in some detail by way of illustration and example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

## EXAMPLES

### Example 1. Identification of mis-TTR Monoclonal Antibodies

**[00228]** Conformationally-specific monoclonal antibodies against monomeric, mis-folded, fibril, or aggregated forms of TTR (mis-TTR) were generated, screened, expressed, and purified as described in Materials and Methods (a-d). In order to generate mis-TTR monoclonal antibodies, the crystal structure of human tetrameric TTR was examined to find regions of the protein that are buried in the tetramer, but exposed upon dissociation of the tetramer into its monomeric subunits. The region identified was residues 89-97 (EHAEEVFTA) (SEQ ID NO:35) located within the F strand of TTR and sequestered at the dimer interface of the tetrameric protein. A BLAST search of the protein database did not reveal any other human proteins possessing this sequence.

**[00229]** A peptide comprising this sequence (ggEHAEEVFTAggkg) (SEQ ID NO:36), was synthesized. Capitalized letters represent residues 89-97 of TTR. Lower case letters represent additional linker residues added to increase the solubility of the antigenic peptide and to establish the 9 amino acid fragment as an internal sequence. This peptide was linked to a poly-lysine dendritic core, generating a multiple antigenic peptide immunogen (TTR-MAP) comprising a

core of lysine residues with multiple branches linked to the TTR 89-97 peptide. The antibodies listed in Table 2 were generated against TTR-MAP.

**[00230]** In addition to this multiple antigenic peptide, two other immunogens containing the same TTR fragment were generated by covalently linking similar TTR 89-97 peptides (Ac-cggEHAEEVVFTA-amide (SEQ ID NO:37) and Ac-EHAEEVVFTAcgg-amide) (SEQ ID NO:38) via the N- and C-terminal cysteine residues to keyhole limpet hemocyanin (TTR89-97-N-KLH and TTR89-97-C-KLH).

**[00231]** Following antibody generation, screening, expression, and purification, detailed binding kinetic parameters (association rate ( $k_a$ ), dissociation rate ( $k_d$ ), and binding affinity constant ( $K_D$ )) were determined for lead mis-TTR antibodies by Surface Plasmon Resonance (SPR) for recombinant human TTR F87M/L110M, as shown in Table 2. Anti-mouse IgG (GE Healthcare) was immobilized on a sensor chip C5 (lacking dextran chains) via amine coupling following the instructions provided in the GE Healthcare anti-mouse kit, and mis-TTR mAbs were captured to a level to ensure a maximum binding of analyte of 30-50 RU. Various concentrations of analyte (recombinant human TTR F87M/L110M) were passed over the captured ligand at 30  $\mu$ l/min in running buffer (HBS + 0.05% P-20, 1 mg/mL BSA) in 3-fold dilutions. For each concentration, the reaction proceeded for a time frame allowing for the higher analyte concentrations to reach equilibrium during association, as well as at least 10% of signal to decay during dissociation. At least one concentration (not the highest or lowest) was run in duplicate. Concentration ranges of analyte were selected based on preliminary experimentation to span at least 10-fold above  $K_D$  to 10-fold below  $K_D$ .

**[00232]** The results of SPR analysis of lead mis-TTR mAbs is shown in Table 2 below.

**Table 2****SPR Analysis of Lead mis-TTR Antibodies Binding to Human TTR (F87M/L110M)**

mAb	$k_a(1/Ms)$	$k_d(1/s)$	$K_D(M)$	$R_{max}$
9D5	2.715E +4	4.930E-4	1.816E-8	31.55
14G8	2.880E+4	5.358E-4	1.861E-8	27.13
5A1	6.107E+4	4.693E-4	7.684E-9	30.98
6C1	4.607E+4	4.151E-4	9.010E-9	26.32

**Example 2. Binding of mis-TTR Antibodies to TTR Antigen**

**[00233]** Four lead mis-TTR mAbs (9D5, 14G8, 6C1, and 5A1) were assayed by ELISA at concentrations ranging from 0.31 to 2.5  $\mu g/ml$  using both pH4.0-treated TTR (pH4-TTR) and native TTR as the coating antigen. TTR antigen preparation and ELISA protocols are described elsewhere in Materials and Methods (e-g).

The resulting binding curves and tabulated  $K_d$  and  $B_{max}$  values are shown in Figure 3 and Table 3 below. The results in Figure 3 are presented in arbitrary units (a.u.) on the y-axis. All mAbs showed significant binding to pH4-TTR with  $K_d$  values ranging from 16 nM (6C1) to 282 nM (9D5).  $B_{max}$  values for binding to pH4-TTR ranged from a low of 0.65 a.u. (14G8) to a high of 2.02 (9D5). In contrast to the binding to pH4-TTR, none of the antibodies showed significant binding to native TTR, indicating that all TTR antibodies generated were specific for non-native forms of TTR.

**Table 3****ELISA Analysis of Lead mis-TTR Antibodies Binding to pH4-TTR**

mAb	$K_d$ (nM)	$B_{max}$ (a.u.)
<b>9D5</b>	282	2.02
<b>14G8</b>	108	0.65
<b>6C1</b>	16	1.07
<b>5A1</b>	23	1.61

Example 3. Analysis of mis-TTR Antibodies by SDS-PAGE and Native-PAGE

**[00234]** 9D5 and 14G8 were analyzed by SDS-PAGE/Western to demonstrate specificity of binding toward monomeric/denatured forms of TTR versus native, non-denatured TTR. SDS-PAGE, Native-PAGE, and Western Blot protocols are described elsewhere in the Methods and Materials (h-j).

**[00235]** Non-denatured TTR or pH4-TTR was run on an SDS-PAGE gel alongside heat-denatured TTR and heat-denatured pH4-TTR. After electrophoresis, the gel was Western blotted onto nitrocellulose and stained with TTR mAbs 9D5 and 14G8. Both antibodies only recognized TTR when it was treated at pH4 or when TTR or pH4-TTR was first heat-denatured prior to SDS-PAGE. These 9D5 and 14G8 thus show a specificity for TTR conformers generated either by denaturation of TTR or by treatment of TTR at pH4.

**[00236]** 6C1 and 5A1 along with total TTR mAbs (7G7, 8C3) and the commercially available Sigma polyclonal antibody were also analyzed by SDS-PAGE/Western. Each blot contained stained molecular weight markers, non-denatured TTR, and pH4-TTR.

**[00237]** The stained SDS-PAGE gel showed that the major species present in the non-denatured TTR sample was an ~38 kDa dimer. In contrast, the major component present in the pH4-TTR sample ran as an ~35kDa dimer with a small amount of dimer of an ~15kDa monomer. This dimer ran as a slightly smaller protein than the dimer present in the non-denatured TTR sample, indicating a conformational difference between these two TTR dimer species.

**[00238]** The Western blots of TTR and pH4-TTR using the four mis-TTR antibodies showed that these mAbs do not recognize non-denatured TTR, but do bind to both the denatured monomer and dimer present in the pH4-TTR sample. Thus, the four mis-TTR mAbs (9D5, 14G8, 6C1, and 5A1) show similar specificities for non-native conformations of TTR when analyzed by SDS-PAGE/Westerns.

**[00239]** In contrast to the four mis-TTR mAbs, the two TTR control mAbs, 7G7 and 8C3 generated through immunization of mice with intact TTR recognized all TTR species present in the TTR and pH4-TTR samples, including tetrameric TTR species. Thus unlike the mis-TTR



mAbs, these control mAbs bind TTR but with no conformational specificity. The Sigma polyclonal antibody behaved similarly to the 7G7 and 8C3 control mAbs.

**[00240]** TTR and pH4-TTR were also run on a native gel to see if the four mis-TTR mAbs were capable of showing conformation specificity under non-denaturing gel conditions. On a stained native PAGE gel, TTR ran as an ~35 kDa native dimer with a small amount of tetramer. In contrast, pH4-TTR ran primarily as a high molecular-weight smear with a trace amount of the ~35 kDa dimer. The non-specific Sigma polyclonal antibody recognized all TTR species present in both the TTR and the pH4-TTR sample. In contrast, 9D5 only recognized the high molecular weight TTR species present in the pH4-TTR sample. As observed in the SDS-PAGE/Western study, 9D5 did not recognize any of the native TTR species.

**[00241]** All four mis-TTR mAbs were subsequently analyzed by native-PAGE/Western blot. As expected and similar to 9D5, the other mis-TTR mAbs, 14G8, 6C1, and 5A1, specifically bound to the high molecular weight non-native forms of TTR present in the pH4-TTR sample. None of these antibodies recognized the ~35 kDa native TTR dimer. These results indicate that the four mis-TTR mAbs behave similarly and recognize only non-native TTR species that are conformationally distinct from native TTR.

#### Example 4. Inhibition of TTR Fiber Formation by mis-TTR Antibodies

**[00242]** TTR-Y78F is a TTR variant containing a point mutation at position 78 in the protein sequence that destabilizes the TTR tetramer. With time and under mildly acidic conditions, this TTR variant dissociates into its monomeric subunits which can then go on to aggregate and form fibers capable of binding to thioflavin-T. The extent of fiber formation can thus be monitored by measuring thioflavin-T fluorescence at 480nm. Introduction of a mis-TTR antibody specific for dissociated TTR monomers or aggregates would prevent the assembly of TTR fibers resulting in a decrease in thioflavin-T fluorescence relative to a no-antibody control reaction. Protocols for examining inhibition of TTR fiber formation are described elsewhere in the Materials and Methods (k).

**[00243]** All four mis-TTR antibodies strongly inhibited the formation of thioflavin-T reactive TTR-Y78F fibers relative to the isotype control (the results are shown in Figure 4 and are

presented in arbitrary units (a.u.) on the y-axis). Mis-TTR antibody 5A1 almost completely inhibited fiber formation. These results are consistent with the notion that mis-TTR antibodies bind monomeric and/or aggregated forms of TTR, thereby preventing the formation of TTR fibers.

**[00244]** Table 4 summarizes the characterization data obtained for the set of 4 mis-TTR antibodies (9D5, 14G8, 6C1, and 5A1) that showed good conformational selectivity for non-native forms of TTR. These antibodies had affinities ( $K_D$ ) for pH4-TTR ranging from 14.5 nM (6C1) to 257 nM (9D5) and  $B_{max}$  values ranging from 0.65 a.u. (14G8) to 2.02 (9D5). None of these antibodies recognized native TTR, but did bind to pH4-TTR on an SDS-PAGE/Western and to the high molecular weight TTR aggregates on a native-PAGE/Western. These antibodies also inhibited the formation of TTR fibrils in the fibril formation assay using Thio-T as the read-out.

**Table 4**

**mis-TTR-Y78F mAb Characterization Summary Table**

Clone ID	Sandwich ELISA (pH4-TTR)		Western Blot			% Inh. Fibrils (Thio-T)
	K <sub>D</sub> (nM)	B <sub>max</sub> (OD <sub>450</sub> a.u. )	SDS-PAGE		Native (HMW-TTR)	
			(TTR)	(pH4-TTR)		
9D5	257	2.02	-	+++	+++	83
14G8	98.7	0.65	-	+++	++	65
6C1	14.6	1.07	-	+++	+++	72
5A1	21.3	1.61	-	+++	+++	100

**[00245]** TTR-V122I is a TTR variant containing a single point mutation at position 122 that destabilizes the tetramer. Fibril formation was associated with an increase in ThT fluorescence. Increasing 14G8 mAb concentrations caused a monotonic decrease in ThT fluorescence indicating a substoichiometric inhibition of TTR fibrillation ( $IC_{50}=0.028\pm0.009$  mg/mL;  $n=3$ ;

Figure 4B and Table 4a). The isotype control mAb did not cause inhibition of TTR fibrillation (Figure 4C), thus demonstrating the specificity of 14G8 mediated inhibition.

**[00246]** Comparable substoichiometric IC<sub>50</sub> values determined for 5A1 and 6C1 (Table 4a) suggested analogous mechanisms of fibril inhibition for each of these mis-TTR mAbs. In contrast, 9D5 unexpectedly failed to inhibit TTR-V122I fibril formation, despite showing similar specificity and affinity for non-native TTR. It remains to be explored whether 9D5 is more sensitive to the assay conditions used.

**Table 4a**

**mis-TTR-V122I mAb Characterization Summary Table**

Antibody	IC <sub>50</sub> ± SD (mg/mL)
9D5	No inhibition
14G8	0.028 ± 0.009
6C1	0.048 ± 0.059
5A1	0.015 ± 0.02
EG 27/1	No inhibition

Example 5. Immunohistochemical (IHC) Characterization of ATTR Tissue Using mis-TTR mAbs

**[00247]** The lead mis-TTR mAbs raised to the TTR 89-97 fragment of the transthyretin protein were immunohistochemically tested on fresh frozen and paraffin processed tissue from confirmed TTR cardiac amyloidosis patients. Protocols for obtaining and preparing cardiac tissue samples, immunohistochemistry (IHC), and image analysis, are provided elsewhere in the Materials and Methods (I-o). The antibodies used for IHC are described in Table 5.

**Table 5****Antibodies Used for Immunohistochemical Characterization**

<b>Antibody</b>	<b>Antibody Type</b>	<b>Vendor</b>	<b>Stain Cardiac Tissue</b>	<b>Concentration</b>
14G8	mis-TTR	Prothena Biosciences	Yes	0.5 µg/mL
9D5	mis-TTR	Prothena Biosciences	Yes	0.5 µg/mL
6C1	mis-TTR	Prothena Biosciences	Yes	0.5 µg/mL
5A1	mis-TTR	Prothena Biosciences	Yes	0.5 µg/mL
7G7	TTR	Prothena Biosciences	Yes	0.5 µg/mL
6F10	Isotype Control	Prothena Biosciences	No	0.5 µg/mL
Prealbumin (A0002)	TTR	Dako North America	Yes	1:2,000 & 1:20,000
Kappa Light Chains (A0191)	LC-κ	Dako North America	No	1:8,000
Lamda Light Chains (A0193)	LC-λ	Dako North America	No	1:8,000
Amyloid A (M0759)	AA	Dako North America	No	1:8,000

**[00248]** Cardiac tissue samples were obtained from patients with confirmed diagnoses of ATTR mutations. Demographics for cases examined immunohistochemically were as follows and are summarized in Table 6: FAC = familial amyloidotic cardiomyopathy; FAP = familial amyloidotic polyneuropathy; 1° AL = light-chain amyloidosis; ATTR = transthyretin-mediated amyloidosis; Unk = Unknown

**Table 6****Immunohistochemical Staining of Cardiac Tissue Samples with mis-TTR Antibodies**

<b>Case</b>	<b>Diagnosis</b>	<b>TTR Mutations</b>	<b>Format</b>	<b>Stained with TTR Antibodies?</b>
<b>Patient 1</b>	FAC	Ileu122	Frozen	Yes

<b>Patient 2</b>	FAP	Wild type	Frozen	Yes
<b>Patient 3</b>	FAP	84Ser	Frozen	Yes
<b>Patient 4</b>	FAP	84Ser	Frozen	Yes
<b>Patient 5</b>	1° AL	--	Frozen	No
<b>Patient 6</b>	1° AL	--	Frozen	No
<b>Patient 7</b>	ATTR	10Arg	Frozen	Yes
<b>Patient 8</b>	ATTR	V122I	Frozen	Yes
<b>Patient H1</b>	ATTR	Val122Ile	FFPE	Yes
<b>Patient H2</b>	ATTR	Thr60Ala	FFPE	Yes
<b>Patient H3</b>	ATTR	Thr49Ala	FFPE	Yes
<b>Patient H4</b>	ATTR	Ile84Ser	FFPE	Yes
<b>Patient H5</b>	Unk.	Senile Cardiac	FFPE	Yes
<b>Patient H6</b>	ATTR	Ile84Ser	FFPE	Yes

**[00249]** Mouse monoclonal antibodies (mis-TTR mAbs) raised to the 89-97 fragment of the transthyretin protein were immunohistochemically tested on fresh frozen and paraffin processed tissue from confirmed TTR cardiac amyloidosis patients. Each mis-TTR antibody showed immunoreactivity on ATTR cardiac tissue. Dark staining was observed in deposits throughout the myocardium and the vasculature. When immunoreactivity was compared to staining with Congo Red or Thioflavin-T, the majority of the immunoreactivity in the tissue showed high congruence with Congo red birefringence and Thioflavin T-positive staining. This confirms the beta pleated sheet nature of the TTR amyloid deposited in this tissue. These mis-TTR antibodies also detected pre-amyloid TTR, which were localized to areas of the myocardium that were TTR- immunopositive but Congo red or Thioflavin T-negative. Both the IgG-isotype control antibody and primary antibody omission sections were negative for staining across all tissues tested. Antibodies reactive toward other amyloidogenic proteins (lambda and kappa light chains or amyloid A) were non-reactive toward the ATTR cardiac tissue used in this analysis, indicating that deposits were specifically TTR in nature.

**[00250]** The staining pattern of mis-TTR antibodies were compared to that obtained with a well characterized commercial TTR reference antibody (prealbumin, A0002; Dako; Carpinteria,

CA). The DAKO reference antibody stained the diseased myocardium in the same areas as the mis-TTR antibodies, but produced a more diffuse staining pattern. The DAKO reference antibody did not stain the congophilic TTR amyloid deposits present on the vasculature as strongly as the mis-TTR antibodies.

**[00251]** The mis-TTR antibodies did not stain normal, non-disease tissue. Furthermore, as expected, staining with an isotype control antibody, 6F10 was also negative.

**[00252]** To determine if the reactivity of mis-TTR antibodies was specific for TTR deposits, cross reactivity of these antibodies toward cardiac tissue derived from patients diagnosed with primary AL amyloidosis was examined. As expected, no staining of AL amyloid tissue was observed, confirming that TTR antibodies react specifically toward ATTR diseased tissue.

**[00253]** Cardiac tissue from patients with confirmed diagnoses of senile systemic amyloidosis or from patients with confirmed FAC, or FAP caused by point mutations in the TTR gene also stained positively with 14G8, 9D5, 6C1, and 5A1. These results indicate that mis-TTR antibodies have the ability to recognize TTR deposits in cardiac tissue regardless of the ATTR genotype.

**[00254]** Other non-cardiac tissues known to express TTR were also examined for staining by 14G8, 9D5, 6C1, and 5A1 and compared to the staining obtained using the DAKO reference antibody. As expected, the liver, pancreas and choroid plexus all stained positively for TTR using the Dako reference antibody. In contrast, mis-TTR antibodies only stained the pancreatic alpha cells located in the islets of Langerhans and the choroid plexus, suggesting that some of the TTR localized to these organs are conformationally distinct from TTR expressed in the liver. The lack of mis-TTR mAb immunoreactivity in the liver suggests that the large amount of TTR expressed there is primarily tetrameric, native TTR and does not have the exposed mis-TTR epitope.

Example 6. Analysis of ATTR vs Normal Human Plasma by SDS-PAGE/Western Blot and by Meso Scale Discovery (MSD) Plate Assay

**[00255]** Six plasma samples from patients confirmed for V30M ATTR (Sample #21, #22, #23, #24, #25, #27) and 6 samples (#11, #12, #15, #18, #19, #20) from normal subjects were

obtained from M. Saraiva (Porto University, Portugal). Sample #C6 was a normal human serum sample obtained from a commercial source (BioreclamationIVT). Samples were analyzed by SDS-PAGE and Western blot, or by MesoScale Discovery (MSD) Plate Assay. Protocols for these assays are described elsewhere in the Materials and Methods (p-r). A standard curve was generated for the MSD Plate Assay using 6C1.

**[00256]** In the resulting Western blots using either the 9D5 or 5A1 mis-TTR mAb, differences between normal and TTR-V30M diseased plasma samples were evident. All plasma samples contained an ~14 kDa TTR band that co-migrated with the non-native TTR monomer present in the pH4-TTR reference sample. In general, plasma samples derived from TTR-V30M patients (#21, 22, 23, 24, 25, & 27) had more of this mis-TTR species. In addition, plasma samples derived from V30M patients also contained an ~30kDa band that co-migrates with the non-native TTR dimer present in the reference sample. With the exception of samples #12 and #18, plasma samples derived from normal individuals possessed less of this dimer species.

**[00257]** The resulting Western blots were scanned and the intensities of the combined 9D5- or 5A1-reactive TTR dimer and monomer bands were plotted for each sample (the results are shown in Figure 5A (9D5) and 5B (5A1) and are presented in arbitrary units (a.u.) on the y-axis). With the exception of plasma samples #15 and #18, plasma samples derived from normal individuals (11, 12, 19, and 20) contained less 9D5 reactive dimer and monomer than samples derived from V30M patients (21-25 and 27).

**[00258]** The 12 serum samples analyzed by 9D5 and 5A1 Western blot were also analyzed by MSD plate assay using 6C1 as the mis-TTR capture antibody and the Dako-SulfoTag antibody as the detection antibody. Results of these MSD assays are shown in Figure 6 and are presented in arbitrary units (a.u.) on the y-axis. Samples 11, 12, 15, 18, 19, and 20 represent normal plasma. Samples 21-25 and 27 represent V30M diseased plasma. With the exception of plasma samples #15 and #18, the amount of 6C1-reactive TTR present in plasma samples derived from normal individuals was lower than that observed in plasma from TTR-V30M diseased individuals. The levels of 6C1 reactivity measured by MSD assay correlated very well with the amount of 9D5 reactive dimer and monomer observed above by SDS-PAGE/Western.

**[00259]** In order to determine the concentration of the reactive TTR species present in plasma samples, the same samples were re-assayed using 6C1 as the capture antibody and 8C3-SulfoTag as the detection antibody. MSD signals were converted to ng/ml concentrations of reactive TTR species using the TTR F87M/L110M standard curve generated above. Based on this analysis, the average concentration of 6C1-reactive TTR present in the control samples was 271 +/- 185 ng/ml. In contrast, the average concentration of reactive TTR present in the V30M diseased plasma samples was higher, at 331 +/- 95 ng/ml. Taken together, these MSD results suggest that mis-TTR antibodies are capable of distinguishing between ATTR disease versus normal plasma. This warrants further development of mis-TTR antibodies for use in diagnostic assays of ATTR disease.

Example 7. Design of Humanized 5A1 Antibodies

**[00260]** The starting point or donor antibody for humanization was the mouse antibody 5A1. The heavy chain variable amino acid sequence of mature m5A1 is provided as SEQ ID NO:1. The light chain variable amino acid sequence of mature m5A1 is provided as SEQ ID NO:9. The heavy chain CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:6, 7, and 8, respectively (as defined by Kabat). The light chain CDR1, CDR2, and CDR3 amino acid sequences are provided as SEQ ID NOS:14, 15, and 17, respectively (as defined by Kabat). Kabat numbering is used throughout in this Example.

**[00261]** The variable kappa (Vk) of m5A1 belongs to mouse Kabat subgroup 1, which corresponds to human Kabat subgroup 4. The variable heavy (Vh) of m5A1 belongs to mouse Kabat subgroup 3d, which corresponds to Kabat subgroup 3. See Kabat *et al.* Sequences of Proteins of Immunological Interest, Fifth Edition. NIH Publication No. 91-3242, 1991. The 11-residue CDR-L1 belongs to canonical class 2, the 7-residue CDR-L2 belongs to canonical class 1, and the 9-residue CDR-L3 belongs to canonical class 1 in Vk. See Martin & Thornton, J. Mol. Biol. 263:800-15, 1996. The 10-residue CDR-H1 (composite of Chothia and Kabat CDR-H1, residues 26-35 of Table 7) belongs to canonical class 1, and the 17-residue CDR-H2 belongs to canonical class 1. See Martin & Thornton, J Mol. Biol. 263:800-15, 1996. The CDR-H3 has no canonical classes.



**[00262]** The residues at the interface between the Vk and Vh domains are the ones commonly found, except that 93V in the heavy chain is typically an alanine.

**[00263]** A search was made over the protein sequences in the PDB database (Deshpande *et al.*, Nucleic Acids Res. 33: D233-7, 2005) to find structures which would provide a rough structural model of 5A1. The crystal structure of antibody fab (pdb code 3IU4) (Talavera *et al.*, 2002) was used for Vk structure since it had good resolution (1.75Å), overall sequence similarity to 5A1 Vk, and retained the same canonical structure for the loop as 5A1. A dimeric antibody (pdb code 3LS4) (Niemi *et al.*, 2003) was used for the Vh structure since it had good similarity and resolution (2.0Å) and contained the same canonical structures for CDR-H1 and CDR-H2 as that of 5A1 VH. BioLuminate software (licensed from Schrodinger Inc.) was used to model a rough structure of 5A1.

**[00264]** A search of the non-redundant protein sequence database from NCBI allowed selection of suitable human frameworks into which to graft the murine CDRs. For Vh, human Ig heavy chain AGP01680.1 (GI: 519674190) (SEQ ID NO:3) was chosen (Bowers *et al.*, 2013). It shares the canonical forms of 5A1. For Vk, a human kappa light chain with NCBI accession code BAH04766 (GI: 219566573) was chosen (SEQ ID NO:11) (Kurosawa *et al.*, 2007). It has the same canonical classes for CDR-L1 and L2 as that for the parental Vk.

**[00265]** Two humanized heavy chain variable region variants and two humanized light chain variable region variants were constructed containing different permutations of substitutions (Hu5A1VHv1-2 (SEQ ID NOS:4 and 5, respectively) and Hu5A1VLv1-2 (SEQ ID NOS:12 and 13, respectively)) (Tables 7 and 8). The exemplary humanized Vh and Vk designs, with backmutations and other mutations based on selected human frameworks, are shown in Tables 7 and 8, respectively. The gray-shaded areas in the first column in Tables 7 and 8 indicate the CDRs as defined by Chothia, and the gray-shaded areas in the remaining columns in Tables 7 and 8 indicate the CDRs as defined by Kabat. SEQ ID NOS:4, 5, 12, and 13 contain backmutations and other mutations as shown in Table 9. The amino acids at positions L55, L85, H29, and H93 in Hu5A1VHv1-2 and Hu5A1VLv1-2 are listed in Table 10.

**Table 7**  
**Humanized 5A1 Vh Regions**

**Table 7**  
**Humanized 5A1 Vh Regions**

Chothia residue #	Kabat residue #	Linear residue #	FR or CDR	Murine 5A1 VH (SEQ ID NO:1)	Hu VL Acceptor Fr Acc#AGP01680 (SEQ ID NO:3)	5A1 VHv1 (SEQ ID NO:4)	5A1 VHv2 (SEQ ID NO:5)
1	1	1	Fr1	E	E	E	E
2	2	2	Fr1	V	V	V	V
3	3	3	Fr1	K	Q	Q	Q
4	4	4	Fr1	L	L	L	L
5	5	5	Fr1	V	V	V	V
6	6	6	Fr1	E	E	E	E
7	7	7	Fr1	S	S	S	S
8	8	8	Fr1	G	G	G	G
9	9	9	Fr1	G	G	G	G
10	10	10	Fr1	G	G	G	G
11	11	11	Fr1	L	L	L	L
12	12	12	Fr1	V	I	I	I
13	13	13	Fr1	R	Q	Q	Q
14	14	14	Fr1	P	P	P	P
15	15	15	Fr1	G	G	G	G
16	16	16	Fr1	G	G	G	G
17	17	17	Fr1	S	S	S	S
18	18	18	Fr1	L	L	L	L
19	19	19	Fr1	K	R	R	R
20	20	20	Fr1	L	L	L	L
21	21	21	Fr1	S	S	S	S
22	22	22	Fr1	C	C	C	C
23	23	23	Fr1	A	A	A	A
24	24	24	Fr1	A	A	A	A
25	25	25	Fr1	S	S	S	S
26	26	26	Fr1	G	G	G	G
27	27	27	Fr1	F	F	F	F
28	28	28	Fr1	T	T	T	T
29	29	29	Fr1	F	V	F	F
30	30	30	Fr1	S	S	S	S
31	31	31	CDR-H1	N	S	N	N
32	32	32	CDR-H1	Y	N	Y	Y

**Table 7**  
**Humanized 5A1 Vh Regions**

33	33	33	CDR-H1	A	Y	A	A
34	34	34	CDR-H1	M	M	M	M
35	35	35	CDR-H1	S	S	S	S
36	36	36	Fr2	W	W	W	W
37	37	37	Fr2	V	V	V	V
38	38	38	Fr2	R	R	R	R
39	39	39	Fr2	Q	Q	Q	Q
40	40	40	Fr2	T	A	A	A
41	41	41	Fr2	P	P	P	P
42	42	42	Fr2	E	G	G	G
43	43	43	Fr2	K	K	K	K
44	44	44	Fr2	R	G	G	G
45	45	45	Fr2	L	L	L	L
46	46	46	Fr2	E	E	E	E
47	47	47	Fr2	W	W	W	W
48	48	48	Fr2	V	V	V	V
49	49	49	Fr2	A	S	S	S
50	50	50	CDR-H2	S	V	S	S
51	51	51	CDR-H2	I	I	I	I
52	52	52	CDR-H2	S	Y	S	S
53	53	53	CDR-H2	S	S	S	S
54	54	54	CDR-H2	G	G	G	G
55	55	55	CDR-H2	G	G	G	G
56	56	56	CDR-H2	S	S	S	S
57	57	57	CDR-H2	T	T	T	T
58	58	58	CDR-H2	Y	Y	Y	Y
59	59	59	CDR-H2	Y	Y	Y	Y
60	60	60	CDR-H2	P	A	P	P
61	61	61	CDR-H2	D	D	D	D
62	62	62	CDR-H2	S	S	S	S
63	63	63	CDR-H2	V	V	V	V
64	64	64	CDR-H2	K	K	K	K
65	65	65	CDR-H2	G	G	G	G
66	66	66	Fr3	R	R	R	R
67	67	67	Fr3	F	F	F	F
68	68	68	Fr3	T	T	T	T
69	69	69	Fr3	I	I	I	I

**Table 7**  
**Humanized 5A1 Vh Regions**

70	70	70	Fr3	S	S	S	S
71	71	71	Fr3	R	R	R	R
72	72	72	Fr3	D	D	D	D
73	73	73	Fr3	N	N	N	N
74	74	74	Fr3	A	S	S	S
75	75	75	Fr3	R	K	K	K
76	76	76	Fr3	N	N	N	N
77	77	77	Fr3	I	T	T	T
78	78	78	Fr3	L	L	L	L
79	79	79	Fr3	Y	Y	Y	Y
80	80	80	Fr3	L	L	L	L
81	81	81	Fr3	Q	Q	Q	Q
82	82	82	Fr3	M	M	M	M
82A	82A	83	Fr3	S	N	N	N
82B	82B	84	Fr3	S	S	S	S
82C	82C	85	Fr3	L	L	L	L
83	83	86	Fr3	R	R	R	R
84	84	87	Fr3	S	A	A	A
85	85	88	Fr3	E	E	E	E
86	86	89	Fr3	D	D	D	D
87	87	90	Fr3	T	T	T	T
88	88	91	Fr3	A	A	A	A
89	89	92	Fr3	M	V	V	V
90	90	93	Fr3	Y	Y	Y	Y
91	91	94	Fr3	Y	Y	Y	Y
92	92	95	Fr3	C	C	C	C
93	93	96	Fr3	V	A	V	A
94	94	97	Fr3	R	R	R	R
95	95	98	CDR-H3	Y	S	Y	Y
96	96	99	CDR-H3	Y	V	Y	Y
97	97	100	CDR-H3	Y	V	Y	Y
98	98	101	CDR-H3	G	Q	G	G
99	99	102	CDR-H3	Q	G	Q	Q
100	100	103	CDR-H3	Y	P	Y	Y
100G	100G	104	CDR-H3	F	H	F	F
101	101	105	CDR-H3	D	L	D	D
102	102	106	CDR-H3	F	D	F	F

**Table 7**  
**Humanized 5A1 Vh Regions**

103	103	107	Fr4	W	W	W	W
104	104	108	Fr4	G	G	G	G
105	105	109	Fr4	Q	Q	Q	Q
106	106	110	Fr4	G	G	G	G
107	107	111	Fr4	T	T	T	T
108	108	112	Fr4	A	L	L	L
109	109	113	Fr4	L	V	V	V
110	110	114	Fr4	T	T	T	T
111	111	115	Fr4	V	V	V	V
112	112	116	Fr4	S	S	S	S
113	113	117	Fr4	S	S	S	S

**Table 8**  
**Humanized 5A1 Vk Regions**

Chothia residue #	Kabat residue #	Linear residue #	FR or CDR	Murine 5A1 VL (SEQ ID NO:9)	Hu VL Acceptor Fr Acc#BAH04766 (SEQ ID NO:11)	5A1 VLv1 (SEQ ID NO:12)	5A1 VLv2 (SEQ ID NO:13)
1	1	1	Fr1	D	D	D	D
2	2	2	Fr1	I	I	I	I
3	3	3	Fr1	V	V	V	V
4	4	4	Fr1	M	M	M	M
5	5	5	Fr1	T	T	T	T
6	6	6	Fr1	Q	Q	Q	Q
7	7	7	Fr1	S	S	S	S
8	8	8	Fr1	H	P	P	P
9	9	9	Fr1	K	S	S	S
10	10	10	Fr1	F	S	S	S
11	11	11	Fr1	M	L	L	L
12	12	12	Fr1	S	S	S	S
13	13	13	Fr1	T	A	A	A
14	14	14	Fr1	S	S	S	S
15	15	15	Fr1	V	V	V	V
16	16	16	Fr1	G	G	G	G
17	17	17	Fr1	D	D	D	D

**Table 8**  
**Humanized 5A1 Vk Regions**

18	18	18	Fr1	R	R	R	R
19	19	19	Fr1	V	V	V	V
20	20	20	Fr1	S	T	T	T
21	21	21	Fr1	I	I	I	I
22	22	22	Fr1	T	T	T	T
23	23	23	Fr1	C	C	C	C
24	24	24	CDR-L1	K	R	K	K
25	25	25	CDR-L1	A	A	A	A
26	26	26	CDR-L1	S	S	S	S
27	27	27	CDR-L1	Q	Q	Q	Q
28	28	28	CDR-L1	D	S	D	D
29	29	29	CDR-L1	V	I	V	V
30	30	30	CDR-L1	S	S	S	S
31	31	31	CDR-L1	T	S	T	T
32	32	32	CDR-L1	T	Y	T	T
33	33	33	CDR-L1	V	S	V	V
34	34	34	CDR-L1	A	N	A	A
35	35	35	Fr2	W	W	W	W
36	36	36	Fr2	Y	Y	Y	Y
37	37	37	Fr2	R	Q	Q	Q
38	38	38	Fr2	Q	Q	Q	Q
39	39	39	Fr2	K	K	K	K
40	40	40	Fr2	P	P	P	P
41	41	41	Fr2	G	G	G	G
42	42	42	Fr2	Q	K	K	K
43	43	43	Fr2	S	A	A	A
44	44	44	Fr2	P	P	P	P
45	45	45	Fr2	E	K	K	K
46	46	46	Fr2	L	L	L	L
47	47	47	Fr2	L	L	L	L
48	48	48	Fr2	I	I	I	I
49	49	49	Fr2	Y	Y	Y	Y
50	50	50	CDR-L2	S	A	S	S
51	51	51	CDR-L2	A	A	A	A
52	52	52	CDR-L2	S	S	S	S
53	53	53	CDR-L2	Y	S	Y	Y
54	54	54	CDR-L2	R	L	R	R

**Table 8**  
**Humanized 5A1 Vk Regions**

55	55	55	CDR-L2	C	Q	C	S
56	56	56	CDR-L2	T	S	T	T
57	57	57	Fr3	G	G	G	G
58	58	58	Fr3	V	V	V	V
59	59	59	Fr3	P	P	P	P
60	60	60	Fr3	D	S	S	S
61	61	61	Fr3	R	R	R	R
62	62	62	Fr3	F	F	F	F
63	63	63	Fr3	T	S	S	S
64	64	64	Fr3	G	G	G	G
65	65	65	Fr3	S	S	S	S
66	66	66	Fr3	G	G	G	G
67	67	67	Fr3	S	S	S	S
68	68	68	Fr3	G	G	G	G
69	69	69	Fr3	A	T	T	T
70	70	70	Fr3	D	D	D	D
71	71	71	Fr3	F	F	F	F
72	72	72	Fr3	T	T	T	T
73	73	73	Fr3	F	L	L	L
74	74	74	Fr3	T	T	T	T
75	75	75	Fr3	I	I	I	I
76	76	76	Fr3	S	S	S	S
77	77	77	Fr3	S	S	S	S
78	78	78	Fr3	V	L	L	L
79	79	79	Fr3	Q	Q	Q	Q
80	80	80	Fr3	A	P	P	P
81	81	81	Fr3	E	E	E	E
82	82	82	Fr3	D	D	D	D
83	83	83	Fr3	L	F	F	F
84	84	84	Fr3	A	A	A	A
85	85	85	Fr3	V	T	V	V
86	86	86	Fr3	Y	Y	Y	Y
87	87	87	Fr3	Y	Y	Y	Y
88	88	88	Fr3	C	C	C	C
89	89	89	CDR-L3	Q	Q	Q	Q
90	90	90	CDR-L3	Q	Q	Q	Q
91	91	91	CDR-L3	H	S	H	H

**Table 8**  
**Humanized 5A1 Vk Regions**

92	92	92	CDR-L3	Y	Y	Y	Y
93	93	93	CDR-L3	S	S	S	S
94	94	94	CDR-L3	T	T	T	T
95	95	95	CDR-L3	P	P	P	P
96	96	96	CDR-L3	L	L	L	L
97	97	97	CDR-L3	T	T	T	T
98	98	98	Fr4	F	F	F	F
99	99	99	Fr4	G	G	G	G
100	100	100	Fr4	A	G	G	G
101	101	101	Fr4	G	G	G	G
102	102	102	Fr4	T	T	T	T
103	103	103	Fr4	K	K	K	K
104	104	104	Fr4	L	V	V	V
105	105	105	Fr4	E	E	E	E
106	106	106	Fr4	L	I	I	I
107	107	107	Fr4	K	K	K	K

**Table 9**  
**V<sub>H</sub>, V<sub>L</sub> Backmutations and Other Mutations**

V <sub>H</sub> or V <sub>L</sub> Variant	V <sub>H</sub> or V <sub>L</sub> Exon Acceptor Sequence	Donor Framework Residues	Kabat CDR Residues
Hu5A1VHv1 (SEQ ID NO:4)	NCBI accession code AGP01680 (SEQ ID NO:3)	H29, H93	-
Hu5A1VHv2 (SEQ ID NO:5)	NCBI accession code AGP01680 (SEQ ID NO:3)	H29	-
Hu5A1VLv1 (SEQ ID NO:12)	NCBI accession code BAH04766 (SEQ ID NO:11)	L85	-
Hu5A1VLv2 (SEQ ID NO:13)	NCBI accession code BAH04766 (SEQ ID NO:11)	L85	L55

**Table 10**  
**Kabat Numbering of Framework and Kabat CDR Residues  
for Backmutations and Other Mutations in Humanized 5A1 Antibodies**

Residue	AGP01680 Heavy Chain	BAH0476 6 Light Chain	Mouse 5A1	Hu5A1 VH1	Hu5A1 VH2	Hu5A1 VL1	Hu5A1 VL2
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L55	-	Q	C	-	-	C	S
L85	-	T	V	-	-	V	V
H29	V	-	F	F	F	-	-
H93	A	-	V	V	A	-	-

**[00266]** An alignment of the murine 5A1 Vh sequence (SEQ ID NO:1) with the mouse model sequence (3LS4\_H\_St.pro; SEQ ID NO:2), the human acceptor sequence (AGP01680; SEQ ID NO:3), and the Hu5A1VHv1 and Hu5A1VHv2 sequences (SEQ ID NOS:4 and 5, respectively), is shown in Figure 1. The CDR regions as defined by Kabat are shaded. Positions at which canonical, vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. Examples of vernier/CDR foundation residues include Kabat residues 2, 49, 69, 71, 75, 78, and 94 in Table 7. Examples of canonical/CDR interacting residues include Kabat residues 24, 48, and 73 in Table 7. Examples of interface/packing (VH+VL) residues include Kabat residues 37, 39, 45, 47, 91, 93, and 103 in Table 7.

**[00267]** An alignment of the murine 5A1 Vk sequence (SEQ ID NO:9) with the mouse model sequence (3IU4\_L\_St.pro; SEQ ID NO:10), the human acceptor sequence (BAH04766; SEQ ID NO:11), and the Hu5A1VLv1 and Hu5A1VLv2 sequences (SEQ ID NOS:12 and 13, respectively), is shown in Figure 2. The CDR regions as defined by Kabat are shaded. Positions at which canonical, vernier, or interface residues differ between mouse and human acceptor sequences are candidates for substitution. Examples of vernier/CDR foundation residues include Kabat residues 4, 35, 46, 49, 66, 68, and 69 in Table 8. Examples of canonical/CDR interacting residues include Kabat residues 2, 48, 64, and 71 in Table 8. Examples of interface/packing (VH+VL) residues include Kabat residues 39, 44, 47, 87, and 98 in Table 8.

**[00268]** The rationales for selection of the above positions as candidates for substitution are as follows.

**[00269]** C55S: Cys is unusual at this position in human antibodies. Ser may retain binding affinity with reduced potential for immunogenicity. This substitution is not a back mutation but rather a substitution of a rare human residue at a position for a more common one.

**[00270]** T85V: Although position 85 is not expected to be important for binding, Thr at this position is less common than the mouse residue Val in human antibodies, so substitution of T for V should decrease potential for immunogenicity without affecting binding.

**[00271]** V29F: This residue was backmutated because it residues in the Chothia CDR.

**[00272]** A93V: This residue contributes to the VH-VL interface. Because acceptor and donor residues differ at this position, an A to V backmutation was made in humanized design VHv1.

**[00273]** The two humanized light chain variable region variants and two humanized heavy chain variable region variants are as follows:

**[00274]** Hu5A1VL version 1 (T85V substitution in lowercase):

DIVMTQSPSSLSASVGDRVITITCKASQDVSTTVAWYQQKPGKAPKLLIYSASYRCTGVP  
SRFSGSGSGTDFTLTISLQPEDFA<sub>v</sub>YYCQQHYSTPLTFGGGTKVEIK (SEQ ID NO:12)

**[00275]** Hu5A1VL version 2 (C55S and T85V substitutions in lowercase):

DIVMTQSPSSLSASVGDRVITITCKASQDVSTTVAWYQQKPGKAPKLLIYSASYR<sub>s</sub>TGVPS  
RFSGSGSGTDFTLTISLQPEDFA<sub>v</sub>YYCQQHYSTPLTFGGGTKVEIK (SEQ ID NO:13)

**[00276]** Hu5A1VH version 1 (V29F and A93V backmutations in lowercase):

EVQLVESGGGLIQPGGSLRLSCAASGFT<sub>f</sub>SNYAMSWVRQAPGKGLEWVSSISSGGSTYY  
PDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC<sub>v</sub>RYYYGQYFDWGGQGLTVTVSS  
(SEQ ID NO:4)

**[00277]** Hu5A1VH version 2 (V29F backmutation in lowercase):

EVQLVESGGGLIQPGGSLRLSCAASGFT<sub>f</sub>SNYAMSWVRQAPGKGLEWVSSISSGGSTYY  
PDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYC<sub>A</sub>RYYYGQYFDWGGQGLTVTVSS  
(SEQ ID NO:5)

Example 8. Binding Kinetic Analysis of Humanized 5A1 Antibodies

**[00278]** Binding kinetics of humanized 5A1 antibodies comprising a heavy chain selected from version 2 and a light chain selected from version 2 were characterized by Biacore.

mAb	$k_a(1/MS)$	$k_d(1/s)$	$K_D(M)$	$R_{max}$
Hu-5A1-H2L2	3.766E+5	3.522E-4	9.352E-10	37.69

Example 9. Materials and Methods*a. Antibody Generation Protocol*

**[00279]** Mice were immunized weekly with the antigenic peptides TTR-MAP, TTR89-97-N-KLH or TTR89-97-C-KLH in RIBI adjuvant or monthly in TiterMax adjuvant. Three to four days prior to fusion, selected mice were given a final IV boost with immunogen in saline solution. Spleen were homogenized to prepare splenocytes and fused with SP2/0 myeloma cells using a standard electrofusion protocol. Fused cells in selection media were plated in 96-well plates and screened after 7-10 days.

*b. Antibody Screening Protocol*

**[00280]** Hybridoma selection was based on the following ELISA screen: 96-well ELISA plates were coated with chicken anti-His, 1 µg/mL PBS and incubated for 1 hour. Plates were blocked with of 1% BSA/PBS solution, 200 uL/well for 15 minutes then 0.5 µg/mL pH4-TTR, 50 µL/well was added and incubated for 1 hour. pH4-TTR is TTR that has been subjected to low pH (50 mM sodium acetate, pH 4.0) in order to dissociate/aggregate TTR, exposing the TTR89-97 epitope. Plates were washed twice with TBS-T. Supernatant from fusion plates was added, 50 µL/well and incubated for 1 hour. Plates were washed twice with TBS-T. The detection antibody, goat anti-mouse (IgG1, 2a, 2b, 3 specific)-HRP diluted 1:5,000 in 0.5% BSA/PBS/TBS-T, 50 µL/well was added and incubated for 1 hour. Finally, plates were washed five times with TBS-T and TMB substrate, 100 µL/well was added. After 15 minutes, substrate development was stopped with 2N Sulfuric Acid, 50 µL/well. Plates were read at 450 nm. Wells with an O.D. > 1.0 were selected and cells were transferred to a 24-well plate. After 3 days of growth, clones were counter screened with the above assay to confirm binding, and substituting native TTR for pH4-TTR as a negative counter screen, allowing for selection of clones producing TTR mAbs specific for non-native forms of TTR.

*c. Antibody Expression Protocols*

**[00281]** CMV driven light chain and heavy chain plasmids carrying humanized monoclonal antibody sequences were transfected into CHO-S1 cells (Life Technology). Dual selection was applied to make a selected pool. Conditioned media was assayed for titer, binding and analyzed by SDS-PAGE/Western blotting. Selected pools were used for clone generation using Clonepix system (Molecular Devices). Clones were ranked based on antibody titer. Selected clones were expanded and banked.

**[00282]** The highest producing clone was expanded in shake flasks and the culture was used to inoculate 10–25L Wave bag cultures. A mixture of FreeStyle-CHO, CD OptiCHO and FreeStyle F17 expression media supplemented with Glutamax (media and Glutamax from Life Technology) was used for shake flask as well as for Wave bag cultures. Batch culture was made using a Wave Bioreactor (GE Healthcare) at 37°C, 7% CO<sub>2</sub> under constant agitation. Samples

were drawn periodically to monitor cell number, viability and antibody production. Supplementation with Cell Boost (HyClone) was made if needed. The batch culture was harvested when cell viability starts to decline below 90% (5 -7 days).

*d. Antibody Purification Protocol*

**[00283]** The cell culture was harvested after first allowing the cells in suspension to settle down to the bottom of the Wave bag via gravity at 4°C. Harvested media was clarified through a depth filter (Millistak Pod COHC, Millipore), concentrated 10-fold by tangential flow filtration (Pelicon 2PLC 30K, Millipore) and sterile filtered through a 0.2 µm filter (Opticap XL, Millipore). The concentrated conditioned media was then loaded onto a Protein G Sepharose Fast Flow column (GE Lifesciences) pre-equilibrated in 1xPBS, pH 7.4 using an FPLC (Akta Avant, GE Lifesciences). Unbound proteins were washed off the column with 5-10 column volumes of 1xPBS, pH 7.4 until the OD<sub>280</sub> reached baseline. The bound antibody was eluted from the column with 2 column volumes of IgG Elution Buffer (Thermo Scientific). Elution fractions were collected and pH neutralized with 2M Tris, pH 9.0 (60 µL per 1ml elution).

**[00284]** Antibody-containing fractions were pooled and dialyzed overnight at 4°C against 1xPBS, pH 7.4. The dialyzed sample was then sterilized by ultrafiltration through a 0.2 µm PES filter and stored at 4°C. The final protein concentration was determined by bicinchoninic acid (BCA) using bovine gamma-globulin as the protein standard (Thermo Scientific).

*e. Recombinant TTR Expression and Purification Protocols*

**[00285]** E. coli (BL21-A1) cells were transformed with a pET21a(+) plasmid containing a TTR insert (Met-hTTR-(His)<sub>6</sub> or a TTR variant containing an F87M/L110M double mutation. Cells were grown in 2YT broth containing 100 µg/ml ampicillin. Expression of TTR was induced overnight at 20°C in the presence of 1mM IPTG and 0.05% arabinose.

**[00286]** The cells were collected by centrifugation at 4000 x g for 10 min. and stored at -80°C until used. 10-15g cell pellets were thawed and lysed in 50ml Buffer A (1xPBS containing 500 mM NaCl, 20mM imidazole) by processing through an LV-1 high-shear processor (Microfluidics, Inc.). Lysed cells were centrifuged at 12,000 x g for 15 min, filtered through a

0.2 µm PES filter prior to purification on a His-Trap HP column (GE Lifesciences). After loading, the column was washed with 10 c.v. of Buffer A and eluted with Buffer B (1xPBS with 500 mM NaCl, 500 mM imidazole). Peak fractions corresponding to TTR were collected, dialyzed against 1xPBS and stored at -80°C until used.

*f. TTR Antigen Preparation*

**[00287]** Native TTR antigen was prepared by diluting a concentrated stock of recombinant TTR-6His to a final concentration of 2.5 µg/ml in 1x PBS. pH4-treated TTR was generated by incubating recombinant TTR at a concentration of 0.2 mg/ml in 50mM sodium acetate, pH 3.95 for 72 hours at room temperature. Under these conditions, TTR dissociates into mixture of TTR monomers and aggregated forms that are structurally distinct from native TTR. The pH4-TTR was then diluted to a final concentration of 2.5 µg/ml in 1xPBS immediately before use in the assay. 96-well plates (Costar #3690) were coated at room temperature with 50 µl per well of 1.0 µg/ml chicken-anti-his polyclonal antibody (Abcam #Ab9107) in 1xPBS for 1 hr. The coating solution was discarded and the plate was blocked with a 250 µl/well volume of 1x BSA-containing block buffer diluted in 1xPBS (G-Biosciences #786-193) for 1hr.

*g. ELISA Protocol*

**[00288]** Coated and blocked 96-well plates were treated with 50 µl per well of 2.5 µg/ml TTR antigen (either native TTR or pH4-TTR) for 1hr. at room temperature. The plates were then washed two times with 250 µl per well of wash buffer (1x Tris Buffered Saline containing 0.05% Tween-20). Washed plates were then treated with 50 µl per well of the appropriate anti-TTR monoclonal antibody at concentrations ranging from 0.31 to 2.5 µg/ml, for 1hr.

**[00289]** The treated plates were washed 3 times with 250 µl perwell wash buffer. After washing, the plates were treated for 1 hr. with 50 µl per well of detection antibody comprising a 1:5,000 dilution of peroxide-conjugated goat-anti-mouse (Jackson ImmunoResearch #115-035-164) in 1xPBS. The plate was then washed 3 times prior to the addition of 100 µl per well TMB substrate (Rockland). The HRP reaction was allowed to proceed at room temperature for 15min.

before quenching with a 50  $\mu$ l per well volume of 1N H<sub>2</sub>SO<sub>4</sub>. Spectroscopic absorbance was measured at a wavelength of 450nm.

*h. SDS-PAGE*

**[00290]** Electrophoresis on SDS-polyacrylamide gels was carried out as follows. 0.1-1  $\mu$ g TTR or pH 4.0-TTR in 1xLDS sample buffer (Life Technologies) was loaded onto a 10% NuPAGE bis-tris gel and subjected to electrophoresis in MES buffer at a constant 90V for 105 minutes. After electrophoresis, the gel was either stained in Instant Blue (Expedeon) or transferred to nitrocellulose filters for Western blot analysis.

*i. Native-PAGE*

**[00291]** Electrophoresis on native Tris-glycine gels was carried out as follows. 0.1-1  $\mu$ g TTR or pH 4.0-TTR in 1xTris-glycine sample buffer (Life Technologies) was loaded onto a 10-20% Tris-glycine gel and subjected to electrophoresis in 1x Native Tris-glycine running buffer at a constant 120V for 105 minutes. After electrophoresis, the gel was either stained in Instant Blue (Expedeon) or transferred to nitrocellulose filters for Western blot analysis.

*j. Western Blot*

**[00292]** SDS- or Native- PAGE gels were blotted onto nitrocellulose filter paper (iBlot, P7 Program) and blocked with blocking buffer (Licor) for 30 minutes. The filters were then incubated in 0.5  $\mu$ g/ml primary antibody in blocking buffer for 1 hour at room temperature (or over-night at 4°C), followed by three, 10 minutes washes with 1xTBS. The filters were placed in IRDye 800CW-conjugated goat-anti-mouse secondary diluted 1:20,000 in block buffer. After incubating the filters in secondary antibody solution for 1 hour at room temperature, the filters were washed and imaged on an Odyssey CLx infrared imager (Licor).

*k. TTR Fiber Formation Assay Protocol*

**[00293]** A solution of 3.6  $\mu$ M (0.2mg/ml) TTR-Y78F in 50mM sodium acetate, pH 4.8 was incubated at 37°C for 72 hours in the presence of 1.4  $\mu$ M (0.2mg/ml) mis-TTR antibody or an isotype control. After incubation, a 5X molar excess of thioflavin-T was added to the mixture

and allowed to bind for 30 minutes. Fluorometric measurements were measured at an emissions wavelength of 480nm with an excitation wavelength set at 440nm. The 0% inhibition was set as the fluorescence intensity in the presence of an isotype control antibody (83 a.u.) and the 100% inhibition point was set as the fluorescence in the absence of TTR-Y78F protein (38 a.u.).

*l. Cardiac tissue samples*

**[00294]** Fresh frozen and paraffin-processed blocks of cardiac tissue with confirmed diagnoses of ATTR mutations were obtained from Dr. Merrill Benson at Indiana University. Samples included eight fresh frozen samples and six FFPE samples and each sample was diagnosed with either ATTR or some other cardiac amyloidosis. The diagnosis of the tissue was further confirmed at Prothena via IHC staining with antibodies to kappa and lambda light chains and amyloid A prior to characterization with the TTR antibodies.

*m. Immunohistochemistry*

**[00295]** Immunohistochemistry was performed on lightly paraformaldehyde-fixed, 10 µm slide-mounted cryosections and on 5 µm paraffin sections. The immunoperoxidase method was the principal detection system, which was performed on the Leica Bond Rx (Leica Biosystems, Buffalo Grove, IL) using the Bond Polymer Refine Detection Kit (DS980, Leica Biosystems). The primary antibodies were incubated for one hour (according to concentrations in Table 2.) followed by incubation with anti-mouse and anti-rabbit polymeric HRP-linker antibody conjugates. The staining was visualized with a DAB chromogen, which produced a brown deposit. The slides were counterstained with hematoxylin, dehydrated in an ascending series of alcohols, cleared in xylenes, and coverslipped with CytoSeal 60 (Richard Allen Scientific; Kalamazoo, MI). Negative control consisted of performing the entire immunohistochemical procedure on adjacent sections with a non-immune IgG isotype control or an omission of the primary antibody.

*n. Demonstration of amyloid: Congo Red and Thioflavin T Staining*

**[00296]** Congo red stain was performed to demonstrate TTR amyloid in the tissue using a kit from American MasterTech (Lodi, California). The staining was performed according to the manufacturer's procedure. Slides were stained in the Congo Red solution for 1 hour followed by



differentiation in 1% sodium hydroxide for approximately 15 seconds. The slides were then rinsed in running water, dehydrated through an alcohol series of increasing concentrations, and cleared through three changes of xylenes, and coverslipped with CytoSeal 60.

**[00297]** A modified Thioflavin T staining protocol (Schmidt et al 1995.) was employed to determine the presence of TTR amyloid in the tissue. Briefly, slides were counterstained with a Mayers hematoxylin, rinsed in running water and stained with a filtered solution of 0.015% Thioflavin T (T3516-25G; Sigma-Aldrich, St. Louis, MO) in 50% ethanol for ten minutes. The slides were then rinsed in running water and differentiated in 1% (v/v) acetic acid for 10 minutes and rinsed three times in water. The slides were allowed to air dry before being coverslipped with ProLong Gold (Life Technologies).

*o. Image Analysis*

**[00298]** Slides were imaged with either an Olympus BX61 microscope, Hamamatsu Nanozoomer 2.0HT digital slide scanner, or a Leica SPE spectral confocal system. Images were collected and stored as TIFF files.

*p. Analysis of human plasma samples by SDS-PAGE/Western*

**[00299]** Six plasma samples from patients confirmed for V30M ATTR (Sample #21, #22, #23, #24, #25, #27) and 6 samples (#11, #12, #15, #18, #19, #20) from normal subjects were obtained from M. Saraiva (Porto University, Portugal). Sample #C6 was a normal human serum sample obtained from a commercial source (BioreclamationIVT). These plasma samples were separated by SDS-PAGE and Western blotted with 9D5 as follow. A 1.4  $\mu$ l volume of plasma was diluted 1:8 into 1xLDS sample buffer in the absence of reducing agent (Life Technologies). Samples were subjected to SDS-PAGE separation and Western blotted with 0.5  $\mu$ g/ml 9D5 as described previously.

*q. Analysis of human plasma samples by MesoScale Discovery (MSD) Plate Assay*

**[00300]** 96-well MSD plates were coated with monoclonal antibody 6C1 at a concentration of 4  $\mu$ g/mL in PBS and incubated for 2 hours at room temperature with shaking, or overnight at 4°C. Plates were washed three times with 1xTBST before being blocked with of 3% MSD

Blocker A solution, 150 µL per well for 1 hour shaking. A 30 µl per well volume of human plasma samples diluted 1:10 in a sample buffer comprised of 0.6% globulin-free bovine serum albumin, 1.5 mM monobasic sodium phosphate, 8mM dibasic sodium phosphate, 145 mM sodium chloride, 0.05% Triton X-405, and 0.05% thimerosal was added to the blocked MSD plates for 1 hour. Plates were washed 3 times with 1xTBST. A 50 µl per well volume of 1 µg/ml sulfo-tagged detection antibody (either 8C3 total TTR antibody of the Dako polyclonal antibody) in sample buffer was added for 1 hr. at room temperature with shaking. Plates were washed three times with 1xTBST followed by the addition of 150 µl per well 1X Read Buffer T solution (Meso Scale Discovery). Plates were then read in the MSD Sector imager.

*r. Generation of an MSD Standard Curve*

**[00301]** In order to quantitate the amount of non-native, 6C1-reactive TTR protein present in human plasma samples, a MSD standard curve was generated using recombinant TTR-F87M/L110M as a 6C1-reactive TTR standard. This TTR variant contains two amino acid substitutions that prevent tetramer formation and keeps the protein in the monomer state (Jiang *et al.* (2001) Biochemistry 40, 11442-11452). As such, this TTR variant is recognized by all mis-TTR mAbs and is therefore well-suited for use as a reference standard in the MSD assay.

**[00302]** To generate the standard curve, 96-well MSD plates were coated with mis-TTR antibody 6C1 at a concentration of 4 µg/mL in PBS and incubated for 2 hours at room temperature with shaking, or overnight at 4°C. Plates were washed three times with 1xTBST before being blocked with 3% MSD Blocker A solution, 150 µL per well for 1 hour shaking. The blocked plates were then treated for 1 hour with 50 µl per well of 25 µg/mL TTR-F87M/L110M serially diluted 1:5 with the last dilution being a buffer blank. Plates were washed 3 times with 1xTBST before the addition of a 50 µl per well volume of 1µg/ml SulfoTag-detection antibody (8C3-SulfoTag or Dako pAb-SulfoTag) for 1 hour at room temperature with shaking. Both 8C3 mAb and the Dako antibody were coupled to the SulfoTag and could be used at the detection antibody since they bound to total TTR and were not conformation specific.

**[00303]** After treatment with the detection antibody, plates were washed three times with a 150 µl per well volume of 1xTBST, followed by the addition of 150 µl per well 1x Read Buffer

T (MSD). Plates were read in the MSD Sector imager and a resulting TTR F87M/L110M calibration curve was generated.

Example 10. Evaluation of mis-TTR Antibodies in Transgenic Mouse Model

**[00304]** *In vivo* studies are conducted in a humanized transgenic mouse model V30M hTTR (Inoue et al., (2008) Specific pathogen free conditions prevent transthyretin amyloidosis in mouse models. Transgenic Research 17:817-826) to assess the efficacy of anti-TTR antibodies in the binding and removal of aggregated hTTR.

**[00305]** Transgenic mice are bred using standard procedures and their circulating hTTR levels are assessed by ELISA. Mice with a serum level of 200-400 µg/ml of hTTR are used for subsequent efficacy studies. The first set of studies examine the natural deposition of hTTR in transgenic mice. Detection of hTTR deposits begins at 12 months of age and is repeated every 3-6 months thereafter. Once an acceptable level of aggregates is seen in transgenic mice, efficacy studies are initiated. Animals are divided into three treatment groups (n=10/ group) and treated weekly for four weeks with an IP dose of vehicle, control antibody (isotype control, 10 mpk) or an anti-hTTR antibody (10 mpk). One week after the last treatment the mice are euthanized, tissues collected and processed, and then stained to assess the number and size of remaining TTR deposits. Quantitative methods and statistics are employed to determine the degree of clearance seen among groups.

**[00306]** In an alternative approach, hTTR aggregates are prepared *in vitro* and then injected into the kidney of transgenic mice to seed the deposition of new aggregates. Applicant has determined that the injection of these preparations can expedite the deposition of new aggregates in a predictable manner. Based on these findings, animals are sedated, the left kidney exposed and pre-aggregated hTTR material injected into the cortex of the kidney. After a suitable recovery period, mice are divided into three treatment groups (n=10/ group) and treated weekly for four-eight weeks with an IP dose of vehicle, control antibody (isotype control, 10 mpk) or an anti-hTTR antibody (10 mpk). One week after the last treatment the mice are euthanized, the kidneys collected and processed, and then stained to assess the number and size of TTR deposits. Quantitative methods and statistics are employed to determine the degree of change seen among groups.

Example 11. Evaluation of mis-TTR Antibodies in a Matrigel Implant Model

**[00307]** Applicant has determined that pre-aggregated hTTR can be suspended in Matrigel (BD Bioscience, Cat #354263), allowed to solidify and then placed subcutaneously in mice. At four weeks post implantation, the Matrigel implant maintained its structure and the aggregated hTTR was still present within the implant. Moreover, the implant was well tolerated by the mice and anti-hTTR antibodies were able to penetrate and bind to the aggregates suspended in the Matrigel. Based on these findings, an antibody efficacy study is conducted. Animals are sedated and an implant containing pre-aggregated hTTR suspended in Matrigel placed subcutaneously in mice. After a suitable recovery period, mice are divided into three treatment groups (n=10/group) and treated weekly, for two-four weeks with an IP dose of vehicle, control antibody (isotype control, 10 mpk) or an anti-hTTR antibody (10 mpk). After the last treatment, the mice are euthanized, the skin containing the implant collected and processed, and then the amount of TTR deposits remaining assessed using histological and/ or biochemical methods. Quantitative analysis and statistics are employed to determine the degree of clearance seen among groups.

**[00308]**

**WHAT IS CLAIMED IS:**

1. An antibody that specifically binds transthyretin comprising a mature heavy chain variable region comprising three Kabat heavy chain CDRs, CDR-H1 of SEQ ID NO:6, CDR-H2 of SEQ ID NO:7, and CDR-H3 of SEQ ID NO:8, and a mature light chain variable region comprising three Kabat light chain CDRs, CDR-L1 of SEQ ID NO:14, CDR-L2 of SEQ ID NO:15 or SEQ ID NO:16, and CDR-L3 of SEQ ID NO:17.

2. The antibody of claim 1, comprising:

(a) CDR-H1 of SEQ ID NO:6, CDR-H2 of SEQ ID NO:7, and CDR-H3 of SEQ ID NO:8, and CDR-L1 of SEQ ID NO:14, CDR-L2 of SEQ ID NO:15, and CDR-L3 of SEQ ID NO:17; or

(b) CDR-H1 of SEQ ID NO:6, CDR-H2 of SEQ ID NO:7, and CDR-H3 of SEQ ID NO:8, and CDR-L1 of SEQ ID NO:14, CDR-L2 of SEQ ID NO:16, and CDR-L3 of SEQ ID NO:17.

3. The antibody of claim 2, comprising CDR-H1 of SEQ ID NO:6, CDR-H2 of SEQ ID NO:7, and CDR-H3 of SEQ ID NO:8, and CDR-L1 of SEQ ID NO:14, CDR-L2 of SEQ ID NO:16, and CDR-L3 of SEQ ID NO:17.

4. The antibody of claim 1 or 2 wherein the heavy chain CDR-H1 is a composite Kabat-Chothia CDR-H1 of SEQ ID NO:52.

5. The antibody of any one of the preceding claims that is a monoclonal antibody.

6. The antibody of any one of the preceding claims that is a chimeric, humanized, or veneered antibody.

7. The antibody of any one of the preceding claims that has human IgG1 isotype, human IgG2 isotype, or human IgG4 isotype.

8. The humanized antibody of claim 6, comprising a humanized mature heavy chain variable region having an amino acid sequence at least 90% identical to SEQ ID

NO:4 or 5 and a humanized mature light chain variable region having an amino acid sequence at least 90% identical to SEQ ID NO: 12 or 13.

9. The humanized antibody of claim 8, wherein at least one of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V; or

wherein at least two of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V; or

wherein at least three of the following positions is occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V; or

wherein all of the following positions are occupied by the amino acid as specified: position H29 is occupied by F, position H93 is occupied by V, position L55 is occupied by S, and position L85 is occupied by V.

10. The humanized antibody of claim 6, comprising a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:12; or comprising a mature heavy chain variable region of SEQ ID NO:4 and a mature light chain variable region of SEQ ID NO:13; or

comprising a mature heavy chain variable region of SEQ ID NO:5 and a mature light chain variable region of SEQ ID NO:12; or

comprising a mature heavy chain variable region of SEQ ID NO:5 and a mature light chain variable region of SEQ ID NO:13.

11. The humanized antibody of claim 6, comprising a mature heavy chain variable region of SEQ ID NO:5 and a mature light chain variable region of SEQ ID NO:13.

12. The antibody of any one of the preceding claims that is an intact antibody.

13. The antibody of any one of claims 1-11 that is a binding fragment.

14. The antibody of claim 13, wherein the binding fragment is a single-chain antibody, Fab, or F(ab')<sub>2</sub> fragment.

15. The antibody of any one of claims 1-12, wherein the mature light chain variable region is fused to a light chain constant region and the mature heavy chain variable region is fused to a heavy chain constant region.

16. The antibody of claim 15, wherein the mature heavy chain variable region is fused to a heavy chain constant region having the sequence of SEQ ID NO:23 with or without the C-terminal lysine and/or the mature light chain variable region is fused to a light chain constant region having the sequence of SEQ ID NO:25.

17. A pharmaceutical composition comprising the antibody of any one of the preceding claims and a pharmaceutically acceptable carrier.

18. A nucleic acid or nucleic acids encoding the heavy chain and light chain of an antibody as described in any one of the preceding claims.

19. A recombinant expression vector comprising a nucleic acid of claim 18 or recombinant expression vectors comprising the nucleic acids of claim 18.

20. A host cell transformed with the recombinant expression vector(s) of claim 19.

21. A method of humanizing an antibody, the method comprising:

(a) selecting one or more acceptor antibody sequences;

(b) identifying amino acid residues of a mouse antibody to be retained;

(c) synthesizing a nucleic acid encoding a humanized heavy chain comprising CDRs of the mouse antibody heavy chain and a nucleic acid encoding a humanized light chain comprising CDRs of the mouse antibody light chain; and

(d) expressing the nucleic acids in a host cell to produce a humanized antibody;

wherein the mouse antibody comprises a heavy chain variable region having an amino acid sequence of SEQ ID NO:1 and a light chain variable region having an amino acid sequence of SEQ ID NO:9.

22. A method of producing a cell line producing a humanized antibody according to any one of claims 6 to 16, or a chimeric, or a veneered antibody according to any one of claims 6 to 7 or 12 to 16, the method comprising:

- (a) introducing a vector encoding heavy and light chains of the antibody and a selectable marker into cells;
- (b) propagating the cells under conditions to select for cells having increased copy number of the vector;
- (c) isolating single cells from the selected cells; and
- (d) banking cells cloned from a single cell selected based on yield of antibody.

23. A method of treating or effecting prophylaxis of a transthyretin-mediated amyloidosis in a subject, comprising administering to the subject an effective regime of the antibody of any one of claims 1 to 16.

24. Use of the antibody of any one of claims 1 to 16 in the manufacture of a medicament for the treatment or prophylaxis of a transthyretin-mediated amyloidosis in a subject.



m5A1VH	10	20	30	40	
3LS4 H St. pro	EVLVESGGGLVLRPGGSLKLSCAASGFTFSNYAMS				42
AGP01680	EVLVESGGGLVKPGGSLKLSCAASGFTFNMYVMWLQTP				42
Hu5A1VHv1	EVQLVESGGGLIQPGGSLRLSCAASGFTVSSNYMS				42
Hu5A1VHv2	EVQLVESGGGLIQPGGSLRLSCAASGFTFSNYAMS				42
	50	60	70	80	
m5A1VH	KRLWVASISSGGSTYYPDSPVKGRFTISRDNARNILYLQ				84
3LS4 H St. pro	KRLWVASISRGGSTYYPDSPVKGRFTISRDNARNILYLQ				84
AGP01680	KGLEWVSNIYSGGSTYYADSVKGRFTISRDNARKNTLYLQ				84
Hu5A1VHv1	KGLEWVSSISSGGSTYYPDSPVKGRFTISRDNARKNTLYLQ				84
Hu5A1VHv2	KGLEWVSSISSGGSTYYPDSPVKGRFTISRDNARKNTLYLQ				84
	90	100	110		
m5A1VH	LRSEDTAMYYCVRRYYGYQYFDFWGGGTALT				117 (SEQ ID NO:1)
3LS4 H St. pro	LRSEDTAMYYCVRRGTIVAGDVWGAGT				117 (SEQ ID NO:2)
AGP01680	LRAEDTAVYYCARSVVQGPLDWGGGT				117 (SEQ ID NO:3)
Hu5A1VHv1	LRAEDTAVYYCVRRYYGYQYFDFWGGGT				117 (SEQ ID NO:4)
Hu5A1VHv2	LRAEDTAVYYCARYYGYQYFDFWGGGT				117 (SEQ ID NO:5)

FIG. 1

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	10	20	30	40
m5A1VL	DIVMTQSHKFMSTSVGDRVSI	TCKASQDVSTTVA	WYRQKP	40
3IU4 L St.pro	DIVMTQSHKFMSTSVGDRVSI	TCKASQDVSTTVA	WYRQKP	40
BAH04766	DIVMTQSPSSLASVGDRTITC	RASQSISSYSN	WYQKP	40
Hu5A1VLv1	DIVMTQSPSSLASVGDRTITC	RASQDVSTTVA	WYQKP	40
Hu5A1VLv2	DIVMTQSPSSLASVGDRTITC	RASQDVSTTVA	WYQKP	40
m5A1VL	GQSPKLLIYASARYCT	GVPDRFTGSGSGADFT	TISSVQA	80
3IU4 L St.pro	GQSPKLLIYASARYCT	GVPDRFTGSGSGADFT	TISSVQA	80
BAH04766	GKAPKLLIYAASSLQSG	VPSPRFGSGSGTDFTL	TISSLQP	80
Hu5A1VLv1	GKAPKLLIYASARYCT	GVPSPRFGSGSGTDFTL	TISSLQP	80
Hu5A1VLv2	GKAPKLLIYASARYST	GVPSPRFGSGSGTDFTL	TISSLQP	80
m5A1VL	EDLAVYYCQQHYSTPLT	FGAGTKLEIK	107	(SEQ ID NO:9)
3IU4 L St.pro	EDLAVYYCQQHYSTPLT	FGAGTKLEIK	107	(SEQ ID NO:10)
BAH04766	EDFATYYCQQSYSTPLT	FGGGTKVEIK	107	(SEQ ID NO:11)
Hu5A1VLv1	EDFATYYCQQHYSTPLT	FGGGTKVEIK	107	(SEQ ID NO:12)
Hu5A1VLv2	EDFATYYCQQHYSTPLT	FGGGTKVEIK	107	(SEQ ID NO:13)

FIG. 2

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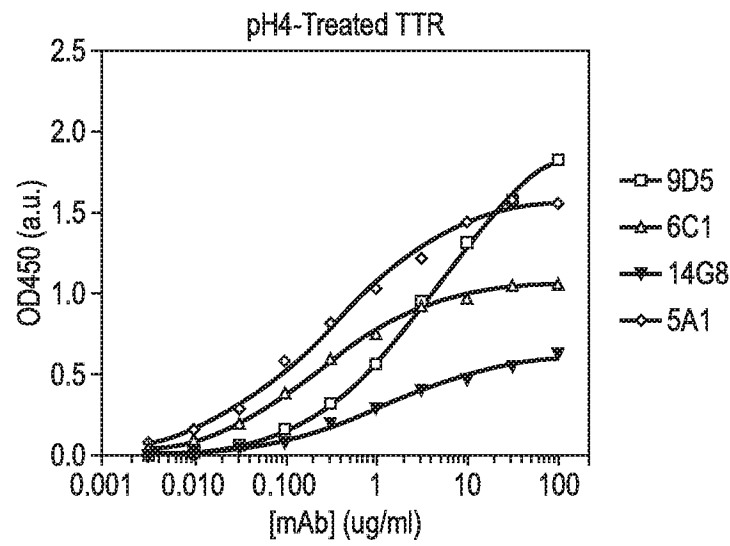


FIG. 3A

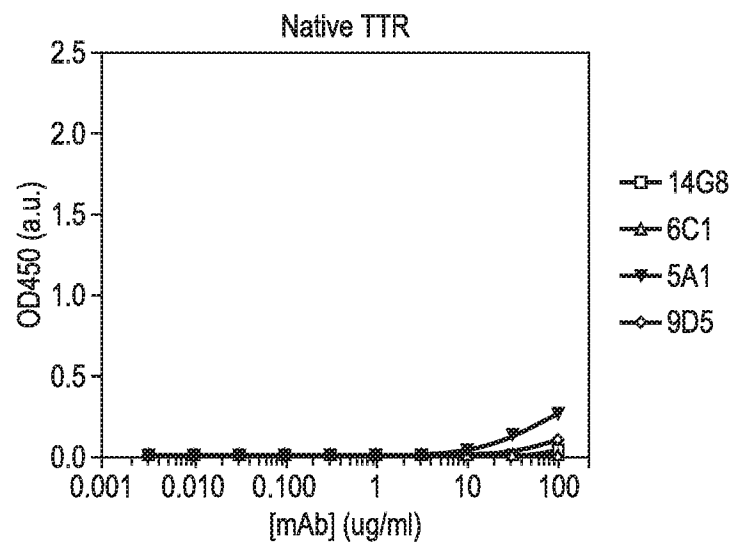
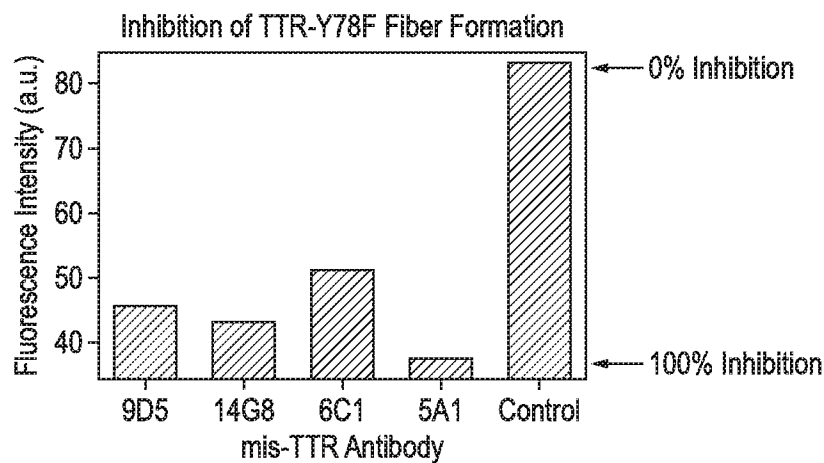
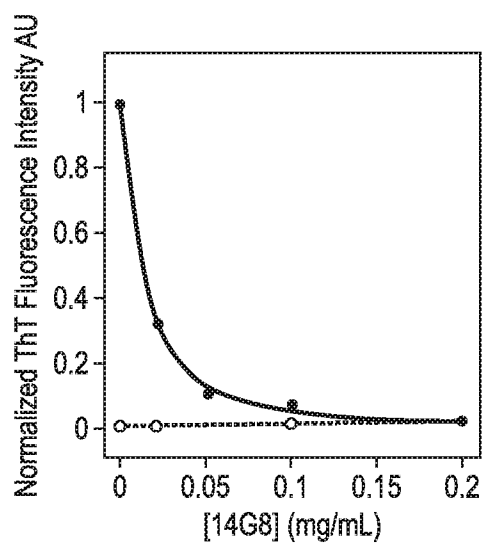
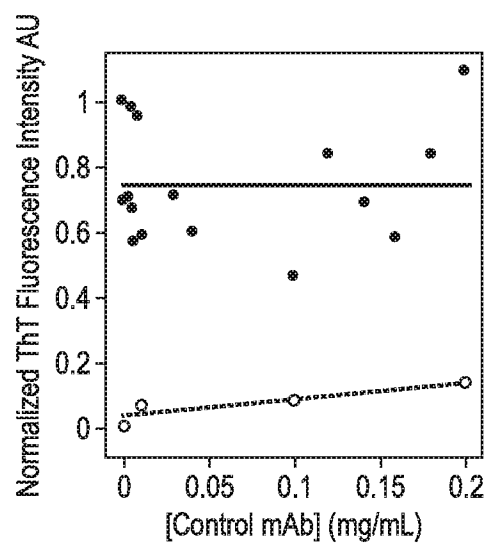
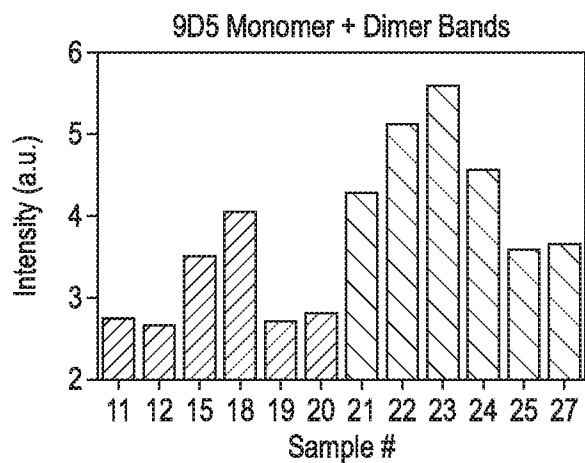
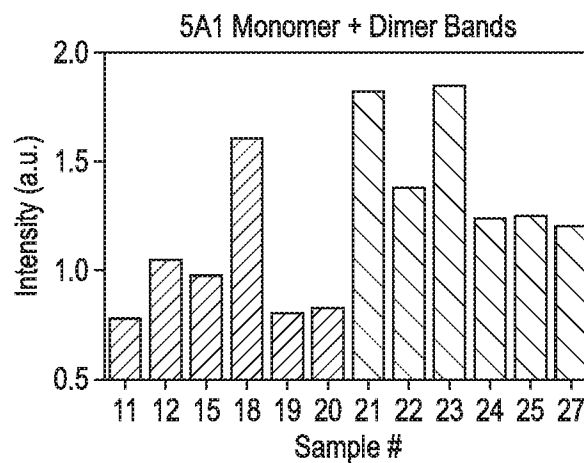


FIG. 3B

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**FIG. 4A****FIG. 4B****FIG. 4C****FIG. 5A****FIG. 5B**

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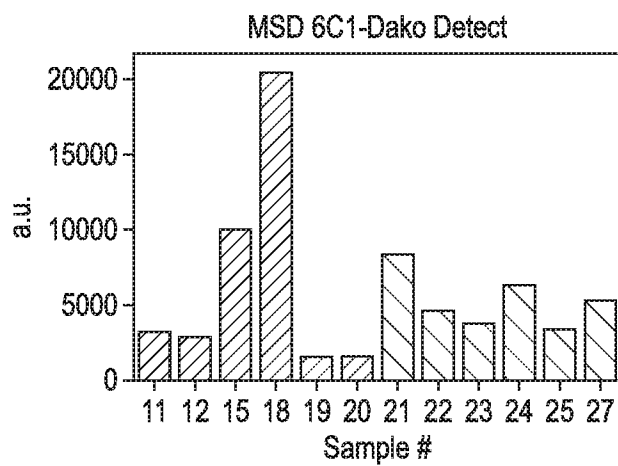


FIG. 6

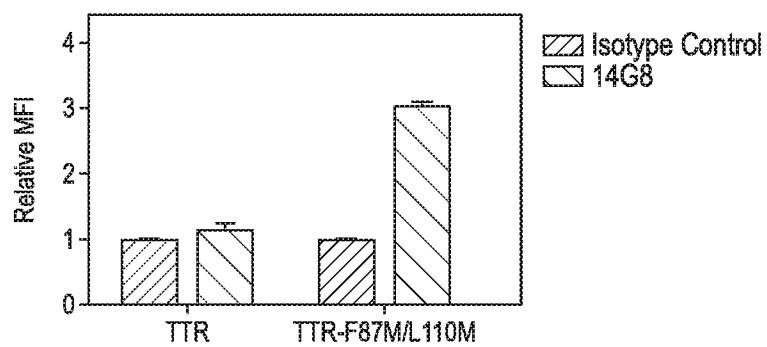


FIG. 7A

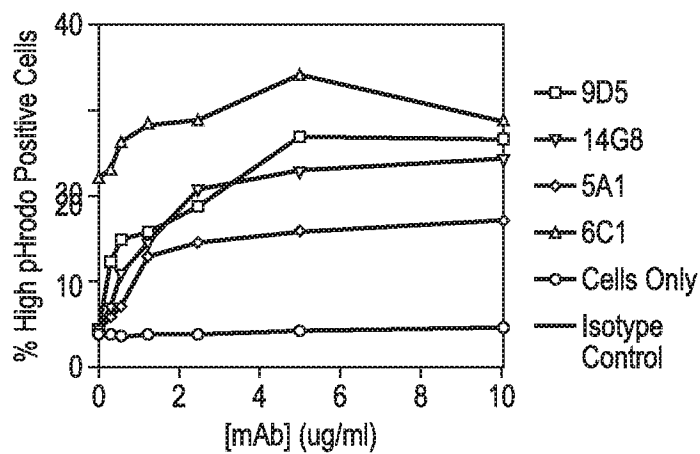


FIG. 7B

## SEQUENCE LISTING

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University Health Network

<120> ANTI -TRANSTHYRETIN ANTIBODIES

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<151> 2015-01-28

<150> 62/266,555

<151> 2015-12-11

<160> 52

<170> FastSEQ for Windows Version 4.0

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Ser	Leu	Lys	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25					30		
Ala	Met	Ser	Trp	Val	Arg	Gln	Thr	Pro	Glu	Lys	Arg	Leu	Glu	Trp	Val
		35					40					45			
Ala	Ser	Ile	Ser	Ser	Gly	Gly	Ser	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys
	50					55					60				
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ala	Arg	Asn	Ile	Leu	Tyr	Leu
65					70					75					80
Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr	Tyr	Cys	Val
				85					90					95	
Arg	Tyr	Tyr	Tyr	Gly	Gln	Tyr	Phe	Asp	Phe	Trp	Gly	Gln	Gly	Thr	Ala
			100					105					110		
Leu	Thr	Val	Ser	Ser											
			115												

<210> 2

<211> 117

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 2

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

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Arg Gly Thr Thr Ile Val Ala Gly Asp Val Trp Gly Ala Gly Thr Thr  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> 3  
 <211> 117  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 3  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Val Ser Ser Asn  
 20 25 30  
 Tyr Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Val Ile Tyr Ser Gly Gly Ser Thr Tyr Tyr Ala Asp Ser Val Lys  
 50 55 60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
 65 70 75 80  
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95  
 Arg Ser Val Val Gln Gly Pro His Leu Asp Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> 4  
 <211> 117  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 4  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ser Ile Ser Ser Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys  
 50 55 60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
 65 70 75 80  
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Val  
 85 90 95  
 Arg Tyr Tyr Tyr Gly Gln Tyr Phe Asp Phe Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> 5  
 <211> 117  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

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<400> 5  
 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Ile Gln Pro Gly Gly  
 1 5 10 15  
 Ser Leu Arg Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe Ser Asn Tyr  
 20 25 30  
 Ala Met Ser Trp Val Arg Gln Ala Pro Gly Lys Gly Leu Glu Trp Val  
 35 40 45  
 Ser Ser Ile Ser Ser Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys  
 50 55 60  
 Gly Arg Phe Thr Ile Ser Arg Asp Asn Ser Lys Asn Thr Leu Tyr Leu  
 65 70 75 80  
 Gln Met Asn Ser Leu Arg Ala Glu Asp Thr Ala Val Tyr Tyr Cys Ala  
 85 90 95  
 Arg Tyr Tyr Tyr Gly Gln Tyr Phe Asp Phe Trp Gly Gln Gly Thr Leu  
 100 105 110  
 Val Thr Val Ser Ser  
 115

<210> 6  
 <211> 5  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 6  
 Asn Tyr Ala Met Ser  
 1 5

<210> 7  
 <211> 16  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 7  
 Ser Ile Ser Ser Gly Gly Ser Thr Tyr Tyr Pro Asp Ser Val Lys Gly  
 1 5 10 15

<210> 8  
 <211> 9  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 8  
 Tyr Tyr Tyr Gly Gln Tyr Phe Asp Phe  
 1 5

<210> 9  
 <211> 107  
 <212> PRT  
 <213> Arti f i c i a l Sequence

<220>  
 <223> Synthesi zed

<400> 9  
 Asp Ile Val Met Thr Gln Ser His Lys Phe Met Ser Thr Ser Val Gly



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1	Asp	Arg	Val	Ser	5	Ile	Thr	Cys	Lys	10	Ala	Ser	Gln	Asp	Val	Ser	15	Thr	Thr
				20						25						30			
	Val	Ala	Trp	Tyr	Arg	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Glu	Leu	Leu	Ile			
			35					40						45					
	Tyr	Ser	Ala	Ser	Tyr	Arg	Cys	Thr	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly			
		50					55					60							
	Ser	Gly	Ser	Gly	Ala	Asp	Phe	Thr	Phe	Thr	Ile	Ser	Ser	Val	Gln	Ala			
65					70						75					80			
	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	His	Tyr	Ser	Thr	Pro	Leu			
				85						90					95				
	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys								
				100					105										

<210> 10  
 <211> 107  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 10	Asp	Ile	Val	Met	Thr	Gln	Ser	His	Lys	Phe	Met	Ser	Thr	Ser	Val	Gly
1					5					10					15	
	Asp	Arg	Val	Ser	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp	Val	Ser	Thr	Ala
			20						25				30			
	Val	Ala	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile
			35					40					45			
	Tyr	Ser	Ala	Ser	Tyr	Arg	Tyr	Thr	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly
		50					55					60				
	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Phe	Thr	Ile	Ser	Ser	Val	Gln	Ala
65					70						75				80	
	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	His	Tyr	Ser	Thr	Pro	Trp
				85					90					95		
	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Leu	Glu	Leu	Lys					
				100					105							

<210> 11  
 <211> 107  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 11	Asp	Ile	Val	Met	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly
1					5					10					15	
	Asp	Arg	Val	Thr	Ile	Thr	Cys	Arg	Ala	Ser	Gln	Ser	Ile	Ser	Ser	Tyr
			20						25				30			
	Ser	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	Leu	Ile
			35					40					45			
	Tyr	Ala	Ala	Ser	Ser	Leu	Gln	Ser	Gly	Val	Pro	Ser	Arg	Phe	Ser	Gly
		50					55					60				
	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	Gln	Pro
65					70						75				80	
	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Tyr	Ser	Thr	Pro	Leu
				85					90					95		
	Thr	Phe	Gly	Gly	Gly	Thr	Lys	Val	Glu	Ile	Lys					
				100					105							

<210> 12  
 <211> 107  
 <212> PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesized

&lt;400&gt; 12

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Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Thr
          20           25           30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
          35           40           45
Tyr Ser Ala Ser Tyr Arg Cys Thr Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65          70          75          80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Leu
          85          90          95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
          100          105

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&lt;210&gt; 13

&lt;211&gt; 107

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesized

&lt;400&gt; 13

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Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly
 1           5           10
Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Asp Val Ser Thr Thr
          20           25           30
Val Ala Trp Tyr Gln Gln Lys Pro Gly Lys Ala Pro Lys Leu Leu Ile
          35           40           45
Tyr Ser Ala Ser Tyr Arg Ser Thr Gly Val Pro Ser Arg Phe Ser Gly
          50           55           60
Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu Gln Pro
65          70          75          80
Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln His Tyr Ser Thr Pro Leu
          85          90          95
Thr Phe Gly Gly Gly Thr Lys Val Glu Ile Lys
          100          105

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&lt;210&gt; 14

&lt;211&gt; 11

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesized

&lt;400&gt; 14

```

Lys Ala Ser Gln Asp Val Ser Thr Thr Val Ala
 1           5           10

```

&lt;210&gt; 15

&lt;211&gt; 7

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesized

<400> 15  
Ser Ala Ser Tyr Arg Cys Thr  
1 5

<210> 16  
<211> 7  
<212> PRT  
<213> Arti fi ci al Sequence

<220>  
<223> Synthesi zed

<400> 16  
Ser Ala Ser Tyr Arg Ser Thr  
1 5

<210> 17  
<211> 9  
<212> PRT  
<213> Arti fi ci al Sequence

<220>  
<223> Synthesi zed

<400> 17  
Gln Gln His Tyr Ser Thr Pro Leu Thr  
1 5

<210> 18  
<211> 408  
<212> DNA  
<213> Arti fi ci al Sequence

<220>  
<223> Synthesi zed

<400> 18  
atgaactttg ggctcagctt gattttcctt gtccttggtt taaaagggtgt ccagtgtgaa 60  
gtgaagctgg tggagtctgg gggaggctta gtgaggcctg gaggggtccct gaaactctcc 120  
tgtgcagcct ctggattcac tticagtaac tatgccatgt ctggggttcg ccagactcca 180  
gagaagaggc tggagtgggt cgcattccatt agtagtggtg gtagcaccta ctatccagac 240  
agtgtgaagg gccgattcac catctccaga gataatgccg ggaacatcct gtacctgcaa 300  
atgagcagtc tgagggtctga ggacacggcc atgtattact gtgtaagata ttactacggc 360  
cagtactttg acttctgggg ccaaggcacc gctctcacag tctcctca 408

<210> 19  
<211> 136  
<212> PRT  
<213> Arti fi ci al Sequence

<220>  
<223> Synthesi zed

<400> 19  
Met Asn Phe Gly Leu Ser Leu Ile Phe Leu Val Leu Val Leu Lys Gly  
1 5 10 15  
Val Gln Cys Glu Val Lys Leu Val Glu Ser Gly Gly Gly Leu Val Arg  
20 25 30  
Pro Gly Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Thr Phe  
35 40 45  
Ser Asn Tyr Ala Met Ser Trp Val Arg Gln Thr Pro Glu Lys Arg Leu  
50 55 60  
Glu Trp Val Ala Ser Ile Ser Ser Gly Gly Ser Thr Tyr Tyr Pro Asp  
65 70 75 80  
Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala Arg Asn Ile

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				85					90					95			
Leu	Tyr	Leu	Gln	Met	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Met	Tyr		
			100					105					110				
Tyr	Cys	Val	Arg	Tyr	Tyr	Tyr	Gly	Gln	Tyr	Phe	Asp	Phe	Trp	Gly	Gln		
		115					120					125					
Gly	Thr	Ala	Leu	Thr	Val	Ser	Ser										
	130					135											

<210> 20  
 <211> 438  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 20  
 atggagtcac atactcaggt ctttgatttc gtgtttctct ggttgctctg tgttgacgga 60  
 gacattgtga tgaccagtc tcacaaattc atgtccacat cagtaggaga cagggtcagc 120  
 atcacctgca aggccagtc ggatgtgagt actactgtag cctggatcgc acagaaacca 180  
 ggacaatctc ctgaactact gatttactcg gcatcctacc ggtgcactgg agtccctgat 240  
 cgcttcactg gcagtggatc tggggcggat ttacatttca ccatcagcag tgtgcaggct 300  
 gaagacctgg cagtttatta ctgtcagcaa cattatagta ctccgctcac gttcgggtgt 360  
 gggaccaagc tggagctgaa acgggctgat gctgcaccaa ctgtatccat cttcccacca 420  
 tccacccgg gatccaag 438

<210> 21  
 <211> 146  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

Met	Glu	Ser	His	Thr	Gln	Val	Phe	Val	Phe	Val	Phe	Leu	Trp	Leu	Ser		
1				5				10					15				
Gly	Val	Asp	Gly	Asp	Ile	Val	Met	Thr	Gln	Ser	His	Lys	Phe	Met	Ser		
		20					25					30					
Thr	Ser	Val	Gly	Asp	Arg	Val	Ser	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Asp		
		35					40					45					
Val	Ser	Thr	Thr	Val	Ala	Trp	Tyr	Arg	Gln	Lys	Pro	Gly	Gln	Ser	Pro		
	50				55						60						
Glu	Leu	Leu	Ile	Tyr	Ser	Ala	Ser	Tyr	Arg	Cys	Thr	Gly	Val	Pro	Asp		
65				70					75					80			
Arg	Phe	Thr	Gly	Ser	Gly	Ser	Gly	Ala	Asp	Phe	Thr	Phe	Thr	Ile	Ser		
			85					90						95			
Ser	Val	Gln	Ala	Glu	Asp	Leu	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	His	Tyr		
		100					105					110					
Ser	Thr	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg		
	115					120					125						
Ala	Asp	Ala	Ala	Pro	Thr	Val	Ser	Ile	Phe	Pro	Pro	Ser	Thr	Pro	Gly		
	130					135					140						
Ser	Lys																
145																	

<210> 22  
 <211> 330  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 22  
 Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys

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1	Ser	Thr	Ser	Gly	5	Thr	Ala	Ala	10	Gly	Cys	Leu	Val	15	Lys	Asp	Tyr
			20						25					30			
Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser		
		35					40					45					
Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser		
	50					55					60						
Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr		
65					70					75					80		
Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys		
			85						90					95			
Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys		
			100					105					110				
Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro		
		115					120					125					
Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys		
	130					135					140						
Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp		
145					150					155					160		
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Val	Lys	Thr	Lys	Pro	Arg	Glu		
			165						170					175			
Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu		
			180					185					190				
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn		
		195					200					205					
Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly		
	210					215					220						
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu		
225					230					235					240		
Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr		
			245						250					255			
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn		
			260					265					270				
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe		
		275					280					285					
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn		
	290					295					300						
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr		
305					310					315					320		
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys								
				325					330								

<210> 23

<211> 330

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 23

Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys
1			5					10					15		
Ser	Thr	Ser	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	
		20					25					30			
Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser
		35					40				45				
Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser
	50					55					60				
Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr
65					70					75				80	
Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys
			85						90				95		
Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys
			100					105					110		
Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro
		115					120					125			

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Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys
Val	130	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn
145						150					155				160
Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu
			165						170					175	
Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu
			180					185					190		
His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn
		195					200					205			
Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys	Gly
	210					215					220				
Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro	Pro	Ser	Arg	Glu	Glu
225					230					235					240
Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu	Val	Lys	Gly	Phe	Tyr
			245						250					255	
Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn	Gly	Gln	Pro	Glu	Asn
			260					265					270		
Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser	Asp	Gly	Ser	Phe	Phe
		275					280					285			
Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg	Trp	Gln	Gln	Gly	Asn
	290					295					300				
Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu	His	Asn	His	Tyr	Thr
305					310					315					320
Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys						
				325					330						

<210> 24

<211> 330

<212> PRT

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 24

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

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Pro Ser Asp Ile<sup>245</sup> Val Glu Trp Glu<sup>250</sup> Ser Asn Gly Gln Pro<sup>255</sup> Glu Asn  
 Asn Tyr Lys Thr<sup>260</sup> Thr Pro Pro Val<sup>265</sup> Leu Asp Ser Asp Gly<sup>270</sup> Ser Phe Phe  
 Leu Tyr<sup>275</sup> Ser Lys Leu Thr Val<sup>280</sup> Asp Lys Ser Arg Trp<sup>285</sup> Gln Gln Gly Asn  
 Val<sup>290</sup> Phe Ser Cys Ser Val<sup>295</sup> Met His Glu Ala Leu<sup>300</sup> His Asn His Tyr Thr<sup>305</sup>  
 Gln Lys Ser Leu Ser<sup>310</sup> Leu Ser Pro Gly Lys<sup>315</sup> Lys<sup>325</sup> Lys<sup>330</sup>

<210> 25  
 <211> 107  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 25  
 Arg Thr Val Ala<sup>5</sup> Ala Pro Ser Val Phe<sup>10</sup> Ile Phe Pro Pro Ser Asp Glu  
 1 Gln Leu Lys Ser Gly Thr Ala Ser Val<sup>15</sup> Val Cys Leu Leu Asn Asn Phe  
 Tyr Pro Arg Glu Ala Lys Val Gln<sup>20</sup> Trp Lys Val Asp Asn<sup>25</sup> Ala Leu Gln  
 Ser Gly<sup>30</sup> Asn Ser Gln Glu Ser<sup>35</sup> Val Thr Glu Gln Asp Ser Lys Asp Ser  
 Thr Tyr Ser Leu Ser Ser<sup>40</sup> Thr Leu Thr Leu Ser<sup>45</sup> Lys Ala Asp Tyr Glu  
 65 Lys His Lys Val Tyr<sup>50</sup> Ala Cys Glu Val Thr<sup>55</sup> His Gln Gly Leu Ser<sup>60</sup> Ser  
 Pro Val Thr Lys<sup>65</sup> Ser Phe Asn Arg Gly<sup>70</sup> Glu Cys  
 100 105

<210> 26  
 <211> 106  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 26  
 Thr Val Ala<sup>5</sup> Ala Pro Ser Val Phe<sup>10</sup> Ile Phe Pro Pro Ser Asp Glu Gln  
 1 Leu Lys Ser Gly Thr Ala Ser Val<sup>15</sup> Val Cys Leu Leu Asn Asn Phe Tyr  
 Pro Arg Glu Ala Lys Val Gln<sup>20</sup> Trp Lys Val Asp Asn<sup>25</sup> Ala Leu Gln Ser  
 Gly Asn Ser Gln Glu Ser<sup>30</sup> Val Thr Glu Gln Asp Ser Lys Asp Ser Thr  
 Tyr Ser Leu Ser Ser<sup>35</sup> Thr Leu Thr Leu Ser<sup>40</sup> Lys Ala Asp Tyr Glu Lys  
 65 His Lys Val Tyr<sup>45</sup> Ala Cys Glu Val Thr<sup>50</sup> His Gln Gly Leu Ser<sup>55</sup> Ser Pro  
 Val Thr Lys Ser<sup>60</sup> Phe Asn Arg Gly<sup>65</sup> Glu Cys  
 100 105

<210> 27  
 <211> 447  
 <212> PRT  
 <213> Artificial Sequence

$\langle 220 \rangle$ 

<223> Synthesi zed

<400> 27

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

<210> 28

<211> 447

<212> PRT

<213> Arti fi ci al Sequence

 $\langle 220 \rangle$



&lt;223&gt; Synthesi zed

&lt;400&gt; 28

Glu	Val	Gln	Leu	Val	Glu	Ser	Gly	Gly	Gly	Leu	Ile	Gln	Pro	Gly	Gly
1				5				10						15	
Ser	Leu	Arg	Leu	Ser	Cys	Ala	Ala	Ser	Gly	Phe	Thr	Phe	Ser	Asn	Tyr
			20					25					30		
Ala	Met	Ser	Trp	Val	Arg	Gln	Ala	Pro	Gly	Lys	Gly	Leu	Glu	Trp	Val
		35					40					45			
Ser	Ser	Ile	Ser	Ser	Gly	Gly	Ser	Thr	Tyr	Tyr	Pro	Asp	Ser	Val	Lys
	50				55						60				
Gly	Arg	Phe	Thr	Ile	Ser	Arg	Asp	Asn	Ser	Lys	Asn	Thr	Leu	Tyr	Leu
65					70					75					80
Gln	Met	Asn	Ser	Leu	Arg	Ala	Glu	Asp	Thr	Ala	Val	Tyr	Tyr	Cys	Ala
				85					90					95	
Arg	Tyr	Tyr	Tyr	Gly	Gln	Tyr	Phe	Asp	Phe	Trp	Gly	Gln	Gly	Thr	Leu
			100					105					110		
Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys	Gly	Pro	Ser	Val	Phe	Pro	Leu
		115					120					125			
Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys
	130					135					140				
Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser
145					150					155					160
Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr	Phe	Pro	Ala	Val	Leu	Gln	Ser
				165					170					175	
Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser
			180					185							
Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn
		195					200					205			
Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro	Lys	Ser	Cys	Asp	Lys	Thr	His
	210					215					220				
Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu	Leu	Leu	Gly	Gly	Pro	Ser	Val
225					230					235					240
Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	Thr	Leu	Met	Ile	Ser	Arg	Thr
				245					250					255	
Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	Val	Ser	His	Glu	Asp	Pro	Glu
			260					265					270		
Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	Val	Glu	Val	His	Asn	Ala	Lys
		275					280					285			
Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	Ser	Thr	Tyr	Arg	Val	Val	Ser
	290					295					300				
Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	Leu	Asn	Gly	Lys	Glu	Tyr	Lys
305					310					315					320
Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	Ala	Pro	Ile	Glu	Lys	Thr	Ile
				325					330					335	
Ser	Lys	Ala	Lys	Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu	Pro
			340					345					350		
Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	Leu
		355					360					365			
Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	Asn
	370					375					380				
Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	Ser
385					390					395					400
Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	Arg
				405					410					415	
Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	Leu
			420					425					430		
His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
		435					440					445			

&lt;210&gt; 29

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Arti f i c i a l   Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 29

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Asp 1 Ile Val Met 5 Thr Gln Ser Pro Ser 10 Ser Leu Ser Ala Ser 15 Val Gly
Asp Arg Val Thr 20 Ile Thr Cys Lys Ala 25 Ser Gln Asp Val 30 Ser Thr Thr
Val Ala Trp 35 Tyr Gln Gln Lys Pro 40 Gly Lys Ala Pro 45 Lys Leu Leu Ile
Tyr Ser 50 Ala Ser Tyr Arg Cys 55 Thr Gly Val Pro Ser 60 Arg Phe Ser Gly
Ser Gly Ser Gly Thr Asp 70 Phe Thr Leu Thr 75 Ile Ser Ser Leu Gln Pro
65 Glu Asp Phe Ala Val 85 Tyr Tyr Cys Gln Gln 90 His Tyr Ser Thr Pro Leu
Thr Phe Gly Gly 100 Gly Thr Lys Val Glu 105 Ile Lys Arg Thr Val 110 Ala Ala
Pro Ser Val Phe 115 Ile Phe Pro Pro 120 Ser Asp Glu Gln Leu Lys Ser Gly
Thr Ala Ser Val Val Cys 135 Leu Leu Asn Asn Phe Tyr 140 Pro Arg Glu Ala
Lys 145 Val Gln Trp Lys Val 150 Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
Glu Ser Val Thr Glu 165 Gln Asp Ser Lys Asp 170 Ser Thr Tyr Ser Leu Ser
Ser Thr Leu Thr 180 Leu Ser Lys Ala Asp 185 Tyr Glu Lys His Lys Val Tyr
Ala Cys Glu Val Thr His Gln Gly 200 Leu Ser Ser Pro Val 205 Thr Lys Ser
Phe Asn Arg Gly Glu Cys
210

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&lt;210&gt; 30

&lt;211&gt; 214

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesized

&lt;400&gt; 30

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Asp 1 Ile Val Met 5 Thr Gln Ser Pro Ser 10 Ser Leu Ser Ala Ser 15 Val Gly
Asp Arg Val Thr 20 Ile Thr Cys Lys Ala 25 Ser Gln Asp Val 30 Ser Thr Thr
Val Ala Trp 35 Tyr Gln Gln Lys Pro 40 Gly Lys Ala Pro 45 Lys Leu Leu Ile
Tyr Ser 50 Ala Ser Tyr Arg Ser 55 Thr Gly Val Pro Ser 60 Arg Phe Ser Gly
Ser Gly Ser Gly Thr Asp 70 Phe Thr Leu Thr 75 Ile Ser Ser Leu Gln Pro
65 Glu Asp Phe Ala Val 85 Tyr Tyr Cys Gln Gln 90 His Tyr Ser Thr Pro Leu
Thr Phe Gly Gly 100 Gly Thr Lys Val Glu 105 Ile Lys Arg Thr Val 110 Ala Ala
Pro Ser Val Phe 115 Ile Phe Pro Pro 120 Ser Asp Glu Gln Leu Lys Ser Gly
Thr Ala Ser Val Val Cys 135 Leu Leu Asn Asn Phe Tyr 140 Pro Arg Glu Ala
Lys 145 Val Gln Trp Lys Val 150 Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln
Glu Ser Val Thr Glu 165 Gln Asp Ser Lys Asp 170 Ser Thr Tyr Ser Leu Ser
Ser Thr Leu Thr 180 Leu Ser Lys Ala Asp 185 Tyr Glu Lys His Lys Val Tyr
Ala Cys Glu Val Thr His Gln Gly 200 Leu Ser Ser Pro Val 205 Thr Lys Ser
Phe Asn Arg Gly Glu Cys

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210

<210> 31  
 <211> 147  
 <212> PRT  
 <213> Homo sapiens

<400> 31  
 Met Ala Ser His Arg Leu Leu Leu Leu Cys Leu Ala Gly Leu Val Phe  
 1 5 10 15  
 Val Ser Glu Ala Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu  
 20 25 30  
 Met Val Lys Val Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val  
 35 40 45  
 Ala Val His Val Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe  
 50 55 60  
 Ala Ser Gly Lys Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr  
 65 70 75 80  
 Glu Glu Glu Phe Val Glu Gly Ile Tyr Lys Val Glu Ile Asp Thr Lys  
 85 90 95  
 Ser Tyr Trp Lys Ala Leu Gly Ile Ser Pro Phe His Glu His Ala Glu  
 100 105 110  
 Val Val Phe Thr Ala Asn Asp Ser Gly Pro Arg Arg Tyr Thr Ile Ala  
 115 120 125  
 Ala Leu Ser Pro Tyr Ser Tyr Ser Thr Thr Ala Val Val Thr Asn  
 130 135 140  
 Pro Lys Glu  
 145

<210> 32  
 <211> 127  
 <212> PRT  
 <213> Homo sapiens

<400> 32  
 Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu Met Val Lys Val  
 1 5 10 15  
 Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val Ala Val His Val  
 20 25 30  
 Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe Ala Ser Gly Lys  
 35 40 45  
 Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr Glu Glu Glu Phe  
 50 55 60  
 Val Glu Gly Ile Tyr Lys Val Glu Ile Asp Thr Lys Ser Tyr Trp Lys  
 65 70 75 80  
 Ala Leu Gly Ile Ser Pro Phe His Glu His Ala Glu Val Val Phe Thr  
 85 90 95  
 Ala Asn Asp Ser Gly Pro Arg Arg Tyr Thr Ile Ala Ala Leu Leu Ser  
 100 105 110  
 Pro Tyr Ser Tyr Ser Thr Thr Ala Val Val Thr Asn Pro Lys Glu  
 115 120 125

<210> 33  
 <211> 127  
 <212> PRT  
 <213> Homo sapiens

<400> 33  
 Gly Pro Thr Gly Thr Gly Glu Ser Lys Cys Pro Leu Met Val Lys Val  
 1 5 10 15  
 Leu Asp Ala Val Arg Gly Ser Pro Ala Ile Asn Val Ala Val His Val  
 20 25 30  
 Phe Arg Lys Ala Ala Asp Asp Thr Trp Glu Pro Phe Ala Ser Gly Lys  
 35 40 45  
 Thr Ser Glu Ser Gly Glu Leu His Gly Leu Thr Thr Glu Glu Glu Phe

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50	Val	Glu	Gly	Ile	Tyr	Lys	55	Val	Glu	Ile	Asp	Thr	Lys	60	Ser	Tyr	Trp	Lys
65	Ala	Leu	Gly	Ile	Ser	Pro	70	Phe	His	Glu	His	Ala	Glu	Val	Val	Phe	Thr	80
				85	Gly	Pro	Arg	Arg	Tyr	90	Thr	Ile	Ala	Ala	Leu	Leu	Ser	95
	Ala	Asn	Asp	100	Ser	Thr	Thr	Ala	105	Val	Val	Thr	Asn	Pro	110	Lys	Glu	115
	Pro	Tyr	Ser	115	Tyr	Ser	Thr	Thr	Ala	120	Val	Val	Thr	Asn	Pro	125	Lys	Glu

<210> 34  
 <211> 138  
 <212> PRT  
 <213> Homo sapiens

<400> 34	Met	Ala	Ser	His	Arg	Leu	Leu	Leu	Leu	Cys	Leu	Ala	Gly	Leu	Val	Phe
1	Val	Ser	Glu	Ala	5	Gly	Pro	Thr	Gly	10	Thr	Gly	Glu	Ser	Lys	Cys
				20	Gly	Pro	Thr	Gly	Thr	25	Gly	Glu	Ser	Lys	Cys	Pro
	Met	Val	Lys	Val	Leu	Asp	Ala	Val	Arg	40	Gly	Ser	Pro	Ala	Ile	Asn
			35	Val	Phe	Arg	Lys	Ala	Ala	45	Asp	Asp	Thr	Trp	Glu	Pro
	Ala	Val	His	Val	Phe	Arg	55	Glu	Ser	60	Gly	Glu	Leu	His	Gly	Leu
	65	Ser	Gly	Lys	Thr	Ser	70	Glu	Ser	75	Gly	Glu	Leu	His	Gly	Leu
	Glu	Glu	Glu	Phe	Val	Glu	85	Gly	Ile	90	Lys	Val	Glu	Ile	Asp	Thr
																95
	Ser	Tyr	Trp	Lys	Ala	Leu	Gly	Ile	Ser	105	Pro	Phe	His	Glu	His	Ala
				100	Thr	Ala	Asn	Asp	Ser	110	Gly	Pro	Arg	Arg	Tyr	Ser
	Val	Val	Phe	115	Thr	Ala	Asn	Asp	Ser	120	Gly	Pro	Arg	Arg	Tyr	Ser
																125
	Thr	Thr	Ala	Val	Val	Thr	Asn	Pro	Lys	Glu						
																130
																135

<210> 35  
 <211> 9  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 35	Glu	His	Ala	Glu	Val	Val	Phe	Thr	Ala
1					5				

<210> 36  
 <211> 15  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Synthesized

<400> 36	Gly	Gly	Glu	His	Ala	Glu	Val	Val	Phe	Thr	Ala	Gly	Gly	Lys	Gly
1					5					10					15

<210> 37  
 <211> 12  
 <212> PRT  
 <213> Artificial Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 37

Cys Gly Gly Glu His Ala Glu Val Val Phe Thr Ala  
 1 5 10

&lt;210&gt; 38

&lt;211&gt; 12

&lt;212&gt; PRT

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 38

Glu His Ala Glu Val Val Phe Thr Ala Cys Gly Gly  
 1 5 10

&lt;210&gt; 39

&lt;211&gt; 990

&lt;212&gt; DNA

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 39

gcctccacca	agggtccatc	ggtcttcccc	ctggcaccct	cctccaagag	cacctctggg	60
ggcacagcgg	ccctgggctg	cctgggtcaag	gactacttcc	ccgaaccggg	gacgggtgtcg	120
tggaaactcag	gcgcccgtgac	cagcggcggtg	cacaccttcc	cggctgtcct	acagtcctca	180
ggactctact	ccctcagcag	cgtgggtgacc	gtgccctcca	gcagcttggg	cacccagacc	240
tacatctgca	acgtgaatca	caagcccagc	aacaccaagg	tggacaagag	agttgagccc	300
aaatcttgtg	acaaaactca	cacatgccca	ccgtgcccag	cacctgaact	cctgggggga	360
ccgtcagtct	tcctcttccc	cccaaaaccc	aaggacaccc	tcatgatctc	ccggaccctt	420
gaggtcacat	gcgtgggtgg	ggacgtgagc	cacgaagacc	ctgaggtcaa	gttcaactgg	480
tacgtggacg	gcgtggagggt	gcataatgcc	aagacaaagc	cgcgaggagga	gcagtacaac	540
agcacgtacc	gtgtggtcag	cgtcctcacc	gtcctgcacc	aggactgggt	gaatggcaag	600
gagtacaagt	gcaagggtctc	caacaaagcc	ctcccagccc	ccatcgagaa	aaccatctcc	660
aaagccaaag	ggcagccccg	agaaccacag	gtgtacaccc	tgcccccatc	ccgggaggag	720
atgaccaaga	accaggtcag	cctgacctgc	ctgggtcaaag	gcttctatcc	cagcgacatc	780
gccgtggagt	gggagagcaa	tgggcagccg	gagaacaact	acaagaccac	gcctcccgtg	840
ctggactccg	acggctcctt	cttcctctat	agcaagctca	ccgtggacaa	gagcagggtg	900
cagcagggga	acgtcttctc	atgctccgtg	atgcatgagg	ctctgcacaa	ccactacacg	960
cagaagagcc	tctccctgtc	cccgggtaaa				990

&lt;210&gt; 40

&lt;211&gt; 321

&lt;212&gt; DNA

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 40

cgaactgtgg	ctgcaccatc	tgtcttcatc	ttcccgccat	ctgatgagca	gttgaaatct	60
ggaactgcct	ctgttgtgtg	cctgctgaat	aacttctatc	ccagagaggc	caaagtacag	120
tggaaagggtg	ataacgccct	ccaatcgggt	aactcccagg	agagtgtcac	agagcaggac	180
agcaaggaca	gcacctacag	cctcagcagc	accctgacgc	tgagcaaagc	agactacgag	240
aaacacaaag	tctacgcctg	cgaagtcacc	catcagggcc	tgagctcgcc	cgtcacaaag	300
agcttcaaca	ggggagagtg	t				321

&lt;210&gt; 41

&lt;211&gt; 318

&lt;212&gt; DNA

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 41

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actgtggctg caccatctgt cttcatcttc ccgccatctg atgagcagtt gaaatctgga 60
actgcctctg ttgtgtgcct gctgaataac ttctatccca gagaggccaa agtacagtgg 120
aaggtggata acgccctcca atcgggtaac tcccaggaga gtgtcacaga gcaggacagc 180
aaggacagca cctacagcct cagcagcacc ctgacgctga gcaaagcaga ctacgagaaa 240
cacaaagtct acgcctgcga agtcacccat cagggcctga gctcgcccgt cacaaagagc 300
ttcaacaggg gagagtgt                                     318

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&lt;210&gt; 42

&lt;211&gt; 19

&lt;212&gt; PRT

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 42

```

Met Asn Phe Gly Leu Ser Leu Ile Phe Leu Val Leu Val Leu Lys Gly
 1           5           10           15
Val Gln Cys

```

&lt;210&gt; 43

&lt;211&gt; 57

&lt;212&gt; DNA

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 43

```

atgaactttg ggctcagctt gattttcctt gtccttgttt taaaagggtgt ccagtgt 57

```

&lt;210&gt; 44

&lt;211&gt; 20

&lt;212&gt; PRT

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 44

```

Met Glu Ser His Thr Gln Val Phe Val Phe Val Phe Leu Trp Leu Ser
 1           5           10           15
Gly Val Asp Gly
                20

```

&lt;210&gt; 45

&lt;211&gt; 60

&lt;212&gt; DNA

&lt;213&gt; Arti fi ci al Sequence

&lt;220&gt;

&lt;223&gt; Synthesi zed

&lt;400&gt; 45

```

atggagtcac atactcaggt ctttgtattc gtgtttctct ggttgtctgg tgttgacgga 60

```

&lt;210&gt; 46

&lt;211&gt; 321

&lt;212&gt; DNA

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 46

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gacattgtga tgaccagtc tcacaaattc atgtccacat cagtaggaga cagggtcagc 60
atcacctgca aggccagtc ggatgtgagt actactgtag cctgggatcg acagaaacca 120
ggacaatctc ctgaactact gatttactcg gcatcctacc ggtgcactgg agtccctgat 180
cgcttcactg gcagtggatc tggggcggat ttactttca ccatcagcag tgtgcaggct 240
gaagacctgg cagtttatta ctgtcagcaa cattatagta ctccgctcac gttcgggtgct 300
gggaccaagc tggagctgaa a                                     321

```

<210> 47

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 47

```

gaagtgaagc tggtaggagtc tgggggaggc ttagtgaggc ctggagggtc cctgaaactc 60
tcctgtgcag cctctggatt cactttcagt aactatgcc a tgtcttgggt tcgccagact 120
ccagagaaga ggctggagtg ggtcgcatcc attagtagtg gtggtagcac ctactatcca 180
gacagtgtga agggccgatt caccatctcc agagataatg ccaggaacat cctgtacctg 240
caaatgagca gtctgaggtc tgaggacacg gccatgtatt actgtgtaag atattactac 300
ggccagtact ttgacttctg gggccaaggc accgctctca cagtctctc a                                     351

```

<210> 48

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 48

```

gagggtgcagc tggtaggagtc cggcggcggc ctgatccagc ccggcgggctc cctgcgcctg 60
tcctgcgccg cctccggctt caccttctcc aactacgcc a tgtcctgggt gcgccaggcc 120
cccggcaagg gcctggagtg ggtgtcctcc atctcctccg gcggctccac ctactacccc 180
gactccgtga agggccgctt caccatctcc cgcgacaact ccaagaacac cctgtacctg 240
cagatgaact ccctgcgcgc cgaggacacc gccgtgtact actgcgtgcg ctactactac 300
ggccagtact tcgacttctg gggccagggc accctggtga ccgtgtcctc a                                     351

```

<210> 49

<211> 351

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthesized

<400> 49

```

gagggtgcagc tggtaggagtc cggcggcggc ctgatccagc ccggcgggctc cctgcgcctg 60
tcctgcgccg cctccggctt caccttctcc aactacgcc a tgtcctgggt gcgccaggcc 120
cccggcaagg gcctggagtg ggtgtcctcc atctcctccg gcggctccac ctactacccc 180
gactccgtga agggccgctt caccatctcc cgcgacaact ccaagaacac cctgtacctg 240
cagatgaact ccctgcgcgc cgaggacacc gccgtgtact actgcgcccg ctactactac 300
ggccagtact tcgacttctg gggccagggc accctggtga ccgtgtcctc a                                     351

```

<210> 50

<211> 321

<212> DNA

<213> Artificial Sequence

<220>

<223> Synthesized

```

<400> 50
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atcacctgca aggcctccca ggacgtgtcc accaccgtgg cctggtagca gcagaagccc 120
ggcaaggccc ccaagctgct gatctactcc gcctcctacc gctgcaccgg cgtgccctcc 180
cgcttctccg gctccggctc cggcaccgac ttcaccctga ccatctcctc cctgcagccc 240
gaggacttcg ccgtgtacta ctgccagcag cactactcca ccccctgac cttcggcggc 300
ggcaccaaag tggagatcaa a 321

```

```

<210> 51
<211> 321
<212> DNA
<213> Artificial Sequence

```

```

<220>
<223> Synthesized

```

```

<400> 51
gacatcgtga tgaccagtc cccctcctcc ctgtccgcct ccgtgggcga ccgcgtgacc 60
atcacctgca aggcctccca ggacgtgtcc accaccgtgg cctggtagca gcagaagccc 120
ggcaaggccc ccaagctgct gatctactcc gcctcctacc gctccaccgg cgtgccctcc 180
cgcttctccg gctccggctc cggcaccgac ttcaccctga ccatctcctc cctgcagccc 240
gaggacttcg ccgtgtacta ctgccagcag cactactcca ccccctgac cttcggcggc 300
ggcaccaaag tggagatcaa a 321

```

```

<210> 52
<211> 10
<212> PRT
<213> Artificial Sequence

```

```

<220>
<223> Synthesized

```

```

<400> 52
Gly Phe Thr Phe Ser Asn Tyr Ala Met Ser
1           5           10

```