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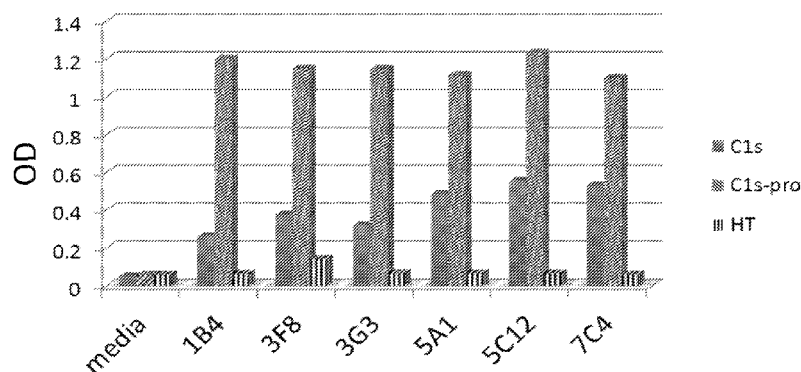
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(54) Title: ANTI-COMPLEMENT FACTOR C1S ANTIBODIES AND USES THEREOF

FIG 1



(57) Abstract: The present invention relates generally to the generation and characterization of neutralizing anti-C1s monoclonal antibodies. The invention further relates to the use of such anti-C1s antibodies in the detection of complement factors of the classical complement activation pathway, such as C1s. Additionally, the antibodies of this disclosure are useful for the diagnosis and treatment of disorders associated with an increased activation of the classical complement pathway, in particular autoimmune disorders and neurodegenerative diseases, including neurodegenerative diseases with synapse loss, such as Alzheimer's Disease. Methods of treatment of autoimmune and neurodegenerative diseases are also provided.



## ANTI-COMPLEMENT FACTOR C1S ANTIBODIES AND USES THEREOF

### CROSS REFERENCES TO RELATED APPLICATIONS

[0001] This application claims the benefit of U.S. Provisional Application No. 61/823,876,  
5 filed May 15, 2013, which is hereby incorporated by reference in its entirety.

### SUBMISSION OF SEQUENCE LISTING ON ASCII TEXT FILE

[0002] The content of the following submission on ASCII text file is incorporated herein by  
reference in its entirety: a computer readable form (CRF) of the Sequence Listing (file name:  
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### BACKGROUND

#### 1. Field

[0003] The present invention relates generally to the generation and characterization of  
15 neutralizing anti-C1s antibodies. The invention further relates to the use of such anti-C1s  
antibodies in the detection of complement factors of the classical complement activation  
pathway, such as C1s. Additionally, the antibodies of this disclosure are useful for the diagnosis  
and treatment of disorders associated with an increased activation of the classical complement  
pathway, in particular autoimmune disorders and neurodegenerative diseases, including  
20 neurodegenerative diseases with synapse loss, such as Alzheimer's Disease. Methods of  
treatment of autoimmune and neurodegenerative diseases are also provided.

#### 2. Description of Related Art

[0004] Many autoimmune diseases and neurodegenerative diseases are associated with  
excessive complement activation. In these cases, complement activation is commonly triggered  
25 through activation of the classical pathway. In autoimmune diseases, the classical pathway of  
complement activation is triggered through binding of autoantibodies to autoantigens, e.g.,  
binding of anti-aquaporin-4 antibodies to aquaporin-4 in patients suffering from Neuromyelitis  
Optica (NMO). In neurodegenerative diseases, factors of the early complement activation  
pathway, such as complement factors C1q, are commonly expressed in neurons. *See, e.g.*, U.S.  
30 Patent Publication Nos. 2012/0195880 and 2012/328601. Neuronal complement expression  
marks neuronal synapses for elimination, resulting in synapse loss and ultimately resulting in  
declining cognitive abilities.

[0005] The cognitive abilities of humans, and especially of patients suffering from neurodegenerative diseases, are highly dependent on synapse formation. The formation of precise neuronal circuits during development is a highly regulated and dynamic process. Excess numbers of synapses are first generated to establish the initial wiring pattern of the brain, but the formation of mature, precise neuronal circuits requires the selective elimination and pruning of specific synapses. Neuronal activity plays a critical role in this refinement phase. However, premature synapse loss in neurodegenerative pathologies results in a loss of neuronal activity, interferes with synaptic pruning, thereby ultimately leading to cognitive decline. In the adult brain, synapse loss often occurs long before the pathology and clinical symptoms in many neurodegenerative diseases. Timely therapeutic intervention to prevent or reduce synapse loss may slow down or prevent progression of clinical symptoms of neurodegenerative diseases. Inhibition of complement activation, especially complement activation through the classical pathway, is commonly viewed as a promising strategy to prevent or reduce synapse loss and corresponding neurodegenerative pathologies.

[0006] Accordingly, inhibition of early complement activation pathways may be a promising therapeutic strategy in neurodegenerative and autoimmune diseases such as Guillain-Barre Syndrome, Myasthenia Gravis, Bullous Pemphigoid, Alzheimer's disease, Parkinson's Disease, Huntington's Disease, Glaucoma, Spinal Muscular Atrophy, Stroke, Traumatic Brain Injury, Multiple Sclerosis, Age-related Macular Degeneration, Neuromyelitis Optica. Thus, there is a need to develop antibodies that inhibit the early stages of complement activation, including the classical complement activation pathway. Specifically, there is a need for neutralizing antibodies, such as neutralizing anti-C1s antibodies, that prevent autoantibodies from triggering the classical pathway of complement activation and that prevent synapse loss resulting from the neuronal expression of complement factors. Moreover, there is a need for antibodies that are useful in the detection of complement factors of the classical complement activation pathway, such as C1s.

[0007] All references cited herein, including patent applications and publications, are hereby incorporated by reference in their entirety.

#### BRIEF SUMMARY

[0008] Provided herein are anti-C1s antibodies and methods of using anti-C1s antibodies. In some embodiments, the anti-C1s antibody binds to a human C1s.

[0009] In certain aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of a monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120351). In other aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of a monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120351). In other aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 and the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of a monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120351).

[0010] In certain aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of a monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120352). In other aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of a monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120352). In other aspects, the present disclosure provides an isolated antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 and the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of a monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof (ATCC Accession No. PTA-120352).

[0011] In some embodiments, the isolated antibody of this disclosure is a murine antibody. In some embodiments the antibody is humanized or a chimeric antibody. In some embodiments, the antibody is murine anti-human C1s monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013, or progeny thereof. In some embodiments, the antibody

is murine anti-human C1s monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 or progeny thereof. An isolated antibody which binds essentially the same C1s epitope as the 5A1 antibody produced by a hybridoma cell line deposited with ATCC on 5/15/2013. In some embodiments, the antibody binds essentially the same C1s epitope as the 5C12 antibody produced by another hybridoma cell line deposited with ATCC on 5/15/2013. In some embodiments, the antibody is of the IgG class, including IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub> isotypes. In some embodiments, the antibody is an antibody fragment, such as an Fab, F(ab')<sub>2</sub>, or Fab' fragment.

**[0012]** In some embodiments, an isolated antibody of this disclosure specifically binds to and neutralizes a biological activity of C1s or the C1s proenzyme. In certain embodiments, the biological activity is C1s binding to C1q, C1s binding to C1r, or C1s binding to C2 or C4. In certain embodiments, the biological activity is the proteolytic enzyme activity of C1s, the conversion of the C1s proenzyme to an active protease, or proteolytic cleavage of C4. In certain embodiments, the biological activity is activation of the classical complement activation pathway, activation of antibody and complement dependent cytotoxicity, or C1F hemolysis.

**[0013]** In other aspects, the present disclosure provides for an isolated host cell comprising a nucleic acid sequence encoding an antibody of this disclosure. In some embodiments, the isolated host cell was deposited with ATCC on 5/15/2013. In other aspects, the present disclosure provides for antibody 5A1, which is produced by an isolated host cell deposited with ATCC on 5/15/2013. In other aspects, the present disclosure provides for antibody 5C12, which is produced by another isolated host cell deposited with ATCC on 5/15/2013.

**[0014]** In other aspects, the present disclosure provides for a pharmaceutical composition comprising an antibody of this disclosure and a pharmaceutically acceptable carrier.

**[0015]** In other aspects, the present disclosure provides a method of treating or preventing an autoimmune or neurodegenerative disease in a subject in need of such treatment, the method comprising the step of administering a therapeutically effective dose of an antibody of any one of the preceding paragraphs. In some embodiments, the autoimmune disease is associated with autoantibodies activating the complement system. In some embodiments, the neurodegenerative disease is associated with the loss of synapses or nerve connections. In some embodiments, the autoimmune or neurodegenerative disease is selected from a group consisting of Guillain-Barre Syndrome, Neuromyelitis Optica, Bullous Pemphigoid, Alzheimer's Disease, Huntington's Disease, Parkinson's Disease, Glaucoma, Spinal Muscular Atrophy, Stroke, Traumatic Brain

Injury, Multiple Sclerosis, Age-related Macular Degeneration, and normal aging. In some embodiments, the disease is associated with C1-dependent pathological synapse loss. In some embodiments, the disease is associated with synapse loss that is dependent on the complement receptor 3(CR3)/C3. In some embodiments, the disease is associated with pathological activity-dependent synaptic pruning. In some embodiments, the disease is associated with synapse phagocytosis by microglia.

**[0016]** In other aspects, the present disclosure provides a diagnostic kit comprising an antibody of any one of the preceding paragraphs. In other aspects, the present disclosure provides a method of detecting synapses in an individual having an autoimmune or

neurodegenerative disease, the method comprising a) administering an antibody of any one of the preceding paragraphs to the individual, and b) detecting antibody bound to synapses, thereby detecting synapses in the individual. In some embodiments, the antibody bound to synapses is detected using imaging techniques selected from the group consisting of positron emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT). In some embodiments, the detection of antibody bound to synapses provides a quantitative measure for the number of synapses in the individual. In some embodiments, the number of synapses in the individual is measured repeatedly over a period of time and a loss of synapses in the individual is detected over time. In some embodiments, the loss of synapses over time is a measure for the efficacy of a treatment for the autoimmune or neurodegenerative disease.

**[0017]** In other aspects, the present disclosure provides a method for detecting synapses in a biological sample, the method comprising a) contacting the biological sample with an antibody of any one of the preceding paragraphs, and b) detecting antibody bound to synapses, thereby detecting synapses in the individual. In some embodiments, the method further comprises c) obtaining the biological sample from the individual. In some embodiments, the biological sample comprises a biopsy specimen, a tissue, or a cell. In some embodiments, the antibody is detected by immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis or immunoprecipitation.

**[0018]** In certain aspects, the present disclosure provides an isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, where the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5A1 produced by a hybridoma cell line with ATCC Accession Number PTA-120351, or

progeny thereof. In other aspects, the present disclosure provides an isolated anti-C1s antibody containing a light chain variable domain and a heavy chain variable domain, where the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5A1 produced by a hybridoma cell line with ATCC Accession Number PTA-120351, or progeny thereof. In other aspects, the present disclosure provides an isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, where the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5A1 produced by a hybridoma cell line with ATCC Accession Number PTA-120351 or progeny thereof, and the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5A1 produced by a hybridoma cell line with ATCC Accession Number PTA-120351 or progeny thereof. In other aspects, the present disclosure provides an isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, where the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC Accession Number PTA-120352, or progeny thereof. In other aspects, the present disclosure provides an isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, where the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC Accession Number PTA-120352, or progeny thereof. In other aspects, the present disclosure provides an isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, where the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5C12 produced by a hybridoma cell line with ATCC Accession Number PTA-120352 or progeny thereof, and the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5C12 produced by a hybridoma cell line with ATCC Accession Number PTA-120352 or progeny thereof.

**[0019]** In other aspects, the present disclosure provides an isolated murine anti-human C1s monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC Accession Number PTA-120351, or progeny thereof. In other aspects, the present disclosure provides an isolated murine anti-human C1s monoclonal antibody 5C12 produced by a hybridoma cell line with ATCC Accession Number PTA-120352 or progeny thereof.

[0020] In other aspects, the present disclosure provides an isolated anti-C1s antibody which binds essentially the same C1s epitope as the antibody 5A1 produced by the hybridoma cell line with ATCC Accession Number PTA-120351 or any of the anti-C1s antibodies derived from antibody 5A1 described herein. In other aspects, the present disclosure provides an isolated anti-C1s antibody which binds essentially the same C1s epitope as the 5C12 antibody produced by the hybridoma cell line with ATCC Accession Number PTA-120352 or any of the anti-C1s antibodies derived from antibody 5A1 described herein.

[0021] In certain embodiments that may be combined with any of the preceding embodiments, the antibody specifically binds to and neutralizes a biological activity of C1s or the C1s proenzyme. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a murine antibody. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is a humanized antibody or a chimeric antibody. In certain embodiments that may be combined with any of the preceding embodiments, the biological activity is (1) C1s binding to C1q, (2) C1s binding to C1r, or (3) C1s binding to C2 or C4. In certain embodiments that may be combined with any of the preceding embodiments, the biological activity is (1) the proteolytic enzyme activity of C1s, (2) the conversion of the C1s proenzyme to an active protease, (3) cleavage of C4, or (4) cleavage of C2. In certain embodiments that may be combined with any of the preceding embodiments, the biological activity is (1) activation of the classical complement activation pathway, (2) activation of antibody and complement dependent cytotoxicity, or (3) C1F hemolysis. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is capable of neutralizing at least 30%, at least 50%, or at least 70% of C1F hemolysis. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is of the IgG class. In certain embodiments that may be combined with any of the preceding embodiments, the antibody has an IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub> isotype. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is an antibody fragment. In certain embodiments that may be combined with any of the preceding embodiments, the antibody fragment is a Fab fragment. In certain embodiments that may be combined with any of the preceding embodiments, the antibody fragment is a F(ab')<sub>2</sub> fragment. In certain embodiments that may be combined with any of the preceding embodiments, the antibody fragment is a Fab' fragment. In certain embodiments that may be combined with any of the preceding embodiments, the antibody fragment specifically binds to and neutralizes a biological activity of C1s or the C1s

proenzyme. In certain embodiments that may be combined with any of the preceding  
embodiments, the antibody fragment has better brain penetration as compared to its  
corresponding full-length antibody. In certain embodiments that may be combined with any of  
the preceding embodiments, the antibody fragment has a shorter half-life as compared to its  
5 corresponding full-length antibody. In certain embodiments that may be combined with any of  
the preceding embodiments, the antibody is a bispecific antibody recognizing a first antigen and  
a second antigen. In certain embodiments that may be combined with any of the preceding  
embodiments, the first antigen is a C1s protein or a C1s proenzyme and the second antigen is an  
antigen facilitating transport across the blood-brain-barrier. In certain embodiments that may be  
10 combined with any of the preceding embodiments, the second antigen is selected from the group  
consisting of transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor  
(IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin  
receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction  
domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an angiopep peptide, and ANG1005.

15 **[0022]** In other aspects, the present disclosure provides an isolated polynucleotide  
comprising a nucleic acid sequence encoding an anti-C1s antibody of any of the preceding  
embodiments. In other aspects, the present disclosure provides an isolated host cell comprising  
the nucleic acid sequence of any of the preceding embodiments. In other aspects, the present  
disclosure provides a hybridoma cell deposited with ATCC on 5/15/2013 with ATCC Accession  
20 Number PTA-120351 or PTA-120352. In other aspects, the present disclosure provides a  
pharmaceutical composition comprising an antibody of any of the preceding embodiments and a  
pharmaceutically acceptable carrier.

**[0023]** In other aspects, the present disclosure provides a method of treating or preventing an  
autoimmune or neurodegenerative disease in an individual in need of such treatment, the method  
25 including the step of administering a therapeutically effective dose of an anti-C1s antibody of any  
of the preceding embodiments. In other aspects, the present disclosure provides an anti-C1s  
antibody of any of the preceding embodiments for use in treating or preventing an autoimmune  
or neurodegenerative disease in an individual in need of such treatment. In other aspects, the  
present disclosure provides use of an anti-C1s antibody of any of the preceding embodiments in  
30 the manufacture of a medicament for treating or preventing an autoimmune or neurodegenerative  
disease in an individual in need of such treatment.

[0024] In certain embodiments that may be combined with any of the preceding embodiments, the autoimmune disease is associated with autoantibodies activating the complement system. In certain embodiments that may be combined with any of the preceding embodiments, the neurodegenerative disease is associated with the loss of synapses or nerve connections. In certain embodiments that may be combined with any of the preceding  
5       embodiments, the autoimmune or neurodegenerative disease is selected from Neuromyelitis Optica, Guillain-Barre Syndrome, Myasthenia Gravis, Bullous Pemphigoid, Alzheimer's Disease, Huntington's Disease, Parkinson's Disease, Glaucoma, Spinal Muscular Atrophy, Stroke, Traumatic Brain Injury, Multiple Sclerosis, Age-related Macular Degeneration, and normal  
10       aging. In certain embodiments that may be combined with any of the preceding embodiments, the disease is associated with C1-dependent pathological synapse loss. In certain embodiments that may be combined with any of the preceding embodiments, the disease is associated with synapse loss that is dependent on the complement receptor 3(CR3)/C3. In certain embodiments that may be combined with any of the preceding embodiments, the disease is associated with  
15       pathological activity-dependent synaptic pruning. In certain embodiments that may be combined with any of the preceding embodiments, the disease is associated with synapse phagocytosis by microglia.

[0025] In other aspects, the present disclosure provides a kit comprising an anti-C1s antibody of any of the preceding embodiments. In some embodiments, the kit is for diagnostic or  
20       therapeutic.

[0026] In other aspects, the present disclosure provides a method of detecting synapses in an individual having an autoimmune or neurodegenerative disease, by a) administering an anti-C1s antibody of any of the preceding embodiments to the individual, and b) detecting antibody bound to synapses, thereby detecting synapses in the individual. In other aspects, the present disclosure  
25       provides an anti-C1s antibody of any of the preceding embodiments for use in detecting synapses in an individual having an autoimmune or neurodegenerative disease. In other aspects, the present disclosure provides use of an anti-C1s antibody of any of the preceding embodiments in the manufacture of a medicament for detecting synapses in an individual having an autoimmune or neurodegenerative disease.

[0027] In certain embodiments that may be combined with any of the preceding  
30       embodiments, the antibody bound to synapses is detected using imaging techniques selected from positron emission tomography (PET), X-ray computed tomography, single-photon emission

computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT). In certain embodiments that may be combined with any of the preceding embodiments, the detection of antibody bound to synapses provides a quantitative measure for the number of synapses in the individual. In certain embodiments that may be combined with any of the preceding embodiments, the number of synapses in the individual is measured repeatedly over a period of time and a loss of synapses in the individual is detected over time. In certain embodiments that may be combined with any of the preceding embodiments, the loss of synapses over time is a measure for the efficacy of a treatment for the autoimmune or neurodegenerative disease.

[0028] In other aspects, the present disclosure provides a method of detecting synapses in a biological sample, by a) contacting the biological sample with an anti-C1s antibody of any of the preceding embodiments, and b) detecting antibody bound to synapses, thereby detecting synapses in the individual.

[0029] In certain embodiments that may be combined with any of the preceding embodiments, the method further includes obtaining the biological sample from the individual. In certain embodiments that may be combined with any of the preceding embodiments, the biological sample contains a biopsy specimen, a tissue, or a cell. In certain embodiments that may be combined with any of the preceding embodiments, the antibody is detected by immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis, or immunoprecipitation.

[0030] It is to be understood that one, some, or all of the properties of the various embodiments described herein may be combined to form other embodiments of the compositions and methods provided herein. These and other aspects of the compositions and methods provided herein will become apparent to one of skill in the art.

## DESCRIPTION OF THE FIGURES

[0031] FIG. 1 illustrates the results of an ELISA screen for antibodies specifically binding human C1s or human C1s proenzyme. The binding assays were conducted either in the absence of an anti-C1s antibody (“media”) or in the presence of one of six anti-C1s antibodies (1B4, 3F8, 3G3, 5A1, 5C12, or 7C4). Left columns show binding signals for anti-C1s antibody binding to the C1s protein; middle columns show binding signals for anti-C1s antibody binding to the C1s proenzyme; right columns show binding signals for anti-C1s antibody binding to the human transferrin (HT) negative control protein.

[0032] FIG. 2 illustrates the C1s neutralizing activities of anti-C1s antibodies in a C1F hemolytic assay. FIG. 2A illustrates the results of assays conducted with six anti-C1s antibodies (1B4, 3F8, 3G3, 5A1, 5C12, or 7C4) in a single-dose format. FIG. 2B shows the results of C1F hemolytic assays conducted with two anti-C1s antibodies (5A1 and 5C12) in a dose-response format.

[0033] FIG. 3 illustrates the C1s neutralizing activities of anti-C1s antibodies in a C4 cleavage assay. The upper panel illustrates the activity of eight anti-C1s antibodies (M241, 3F8, 3A1, 3A2, 2A1, 5C12, 6HK, and 8HK) regarding the inhibition of C4 cleavage at a single concentration. The lower panel illustrates the neutralizing activities of two anti-C1s antibodies (5A1 and 5C12) in a dose-response format.

## DETAILED DESCRIPTION OF THE INVENTION

### General techniques

[0034] The techniques and procedures described or referenced herein are generally well understood and commonly employed using conventional methodology by those skilled in the art, such as, for example, the widely utilized methodologies described in Sambrook et al., *Molecular Cloning: A Laboratory Manual* 3d edition (2001) Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.; *Current Protocols in Molecular Biology* (F.M. Ausubel, et al. eds., (2003)); the series *Methods in Enzymology* (Academic Press, Inc.); *PCR 2: A Practical Approach* (M.J. MacPherson, B.D. Hames and G.R. Taylor eds. (1995)), Harlow and Lane, eds. (1988) *Antibodies, A Laboratory Manual*, and *Animal Cell Culture* (R.I. Freshney, ed. (1987)); *Oligonucleotide Synthesis* (M.J. Gait, ed., 1984); *Methods in Molecular Biology*, Humana Press; *Cell Biology: A Laboratory Notebook* (J.E. Cellis, ed., 1998) Academic Press; *Animal Cell Culture* (R.I. Freshney), ed., 1987); *Introduction to Cell and Tissue Culture* (J.P. Mather and P.E. Roberts, 1998) Plenum Press; *Cell and Tissue Culture: Laboratory Procedures* (A. Doyle, J.B. Griffiths, and D.G. Newell, eds., 1993-8) J. Wiley and Sons; *Handbook of Experimental Immunology* (D.M. Weir and C.C. Blackwell, eds.); *Gene Transfer Vectors for Mammalian Cells* (J.M. Miller and M.P. Calos, eds., 1987); *PCR: The Polymerase Chain Reaction*, (Mullis et al., eds., 1994); *Current Protocols in Immunology* (J.E. Coligan et al., eds., 1991); *Short Protocols in Molecular Biology* (Wiley and Sons, 1999); *Immunobiology* (C.A. Janeway and P. Travers, 1997); *Antibodies* (P. Finch, 1997); *Antibodies: A Practical Approach* (D. Catty., ed., IRL Press, 1988-1989); *Monoclonal Antibodies: A Practical Approach* (P. Shepherd and C. Dean, eds., Oxford University Press, 2000); *Using Antibodies: A Laboratory Manual* (E. Harlow and D.

Lane (Cold Spring Harbor Laboratory Press, 1999); *The Antibodies* (M. Zanetti and J. D. Capra, eds., Harwood Academic Publishers, 1995); and *Cancer: Principles and Practice of Oncology* (V.T. DeVita et al., eds., J.B. Lippincott Company, 1993).

### Definitions

- 5    **[0035]**     As used herein, the term “*preventing*” includes providing prophylaxis with respect to occurrence or recurrence of a particular disease, disorder, or condition in an individual. An individual may be predisposed to, susceptible to a particular disease, disorder, or condition, or at risk of developing such a disease, disorder, or condition, but has not yet been diagnosed with the disease, disorder, or condition.
- 10   **[0036]**     As used herein, an individual “*at risk*” of developing a particular disease, disorder, or condition may or may not have detectable disease or symptoms of disease, and may or may not have displayed detectable disease or symptoms of disease prior to the treatment methods described herein. “At risk” denotes that an individual has one or more risk factors, which are measurable parameters that correlate with development of a particular disease, disorder, or
- 15   condition, as known in the art. An individual having one or more of these risk factors has a higher probability of developing a particular disease, disorder, or condition than an individual without one or more of these risk factors.
- [0037]**     As used herein, the term “*treatment*” refers to clinical intervention designed to alter the natural course of the individual being treated during the course of clinical pathology.
- 20   Desirable effects of treatment include decreasing the rate of progression, ameliorating or palliating the pathological state, and remission or improved prognosis of a particular disease, disorder, or condition. An individual is successfully “treated”, for example, if one or more symptoms associated with a particular disease, disorder, or condition are mitigated or eliminated.
- [0038]**     An “*effective amount*” refers to at least an amount effective, at dosages and for
- 25   periods of time necessary, to achieve the desired therapeutic or prophylactic result. An effective amount can be provided in one or more administrations.
- [0039]**     A “*therapeutically effective amount*” is at least the minimum concentration required to effect a measurable improvement of a particular disease, disorder, or condition. A therapeutically effective amount herein may vary according to factors such as the disease state,
- 30   age, sex, and weight of the patient, and the ability of the anti-C1s antibody to elicit a desired response in the individual. A therapeutically effective amount may also be one in which any

toxic or detrimental effects of the anti-C1s antibody are outweighed by the therapeutically beneficial effects.

[0040] *Chronic* administration refers to administration of the medicament(s) in a continuous as opposed to acute mode, so as to maintain the initial therapeutic effect (activity) for an extended period of time. *Intermittent* administration refers to treatment that is not consecutively done without interruption, but rather is cyclic in nature.

[0041] As used herein, administration *in conjunction* with another compound or composition includes simultaneous administration and/or administration at different times. Administration in conjunction also encompasses administration as a co-formulation or administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of administration.

[0042] An *individual* for purposes of treatment, prevention, or reduction of risk refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sport, or pet animals, such as dogs, horses, rabbits, cattle, pigs, hamsters, gerbils, mice, ferrets, rats, cats, and the like. Preferably, the individual is human.

[0043] As used herein, *autoantibody* means any antibody that recognizes a host antigen. In some embodiments, the host antigen is AQP4. For example, in an individual having NMO, AQP4 activates the classical pathway of complement activation. In the first step of this activation process complement factor C1q binds to the autoantibody-autoantigen-immune complex. Autoantibodies may include naturally occurring antibodies, such as serum antibodies from NMO patients (commonly referred to as NMO-IgG) or monoclonal antibodies, such as rAb-53.

[0044] The term *immunoglobulin* (Ig) is used interchangeably with *antibody* herein. The term *antibody* herein is used in the broadest sense and specifically covers monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies) formed from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

[0045] The basic 4-chain antibody unit is a heterotetrameric glycoprotein composed of two identical light (L) chains and two identical heavy (H) chains. The pairing of a V<sub>H</sub> and V<sub>L</sub> together forms a single antigen-binding site. For the structure and properties of the different classes of antibodies, *see, e.g., Basic and Clinical Immunology*, 8th Ed., Daniel P. Stites, Abba I.

Terr and Tristram G. Parslow (eds.), Appleton & Lange, Norwalk, CT, 1994, page 71 and Chapter 6.

[0046] The L chain from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (“κ”) and lambda (“λ”), based on the amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains (CH), immunoglobulins can be assigned to different classes or isotypes. There are five classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, having heavy chains designated alpha (“α”), delta (“δ”), epsilon (“ε”), gamma (“γ”) and mu (“μ”), respectively. The γ and α classes are further divided into subclasses (isotypes) on the basis of relatively minor differences in the CH sequence and function, *e.g.*, humans express the following subclasses: IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2. The subunit structures and three dimensional configurations of different classes of immunoglobulins are well known and described generally in, for example, Abbas *et al.*, Cellular and Molecular Immunology, 4<sup>th</sup> ed. (W.B. Saunders Co., 2000).

[0047] “*Native antibodies*” are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V<sub>H</sub>) followed by a number of constant domains. Each light chain has a variable domain at one end (V<sub>L</sub>) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light chain and heavy chain variable domains.

[0048] An “*isolated*” antibody, such as an anti-C1s antibody of the present disclosure, is one that has been identified, separated and/or recovered from a component of its production environment (*e.g.*, naturally or recombinantly). Preferably, the isolated polypeptide is free of association with all other contaminant components from its production environment. Contaminant components from its production environment, such as those resulting from recombinant transfected cells, are materials that would typically interfere with research, diagnostic or therapeutic uses for the antibody, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the polypeptide will be

purified: (1) to greater than 95% by weight of antibody as determined by, for example, the Lowry method, and in some embodiments, to greater than 99% by weight; (2) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (3) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Isolated antibody includes the antibody *in situ* within recombinant T-cells since at least one component of the antibody's natural environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by at least one purification step.

[0049] The “*variable region*” or “*variable domain*” of an antibody, such as an anti-C1s antibody of the present disclosure, refers to the amino-terminal domains of the heavy or light chain of the antibody. The variable domains of the heavy chain and light chain may be referred to as “V<sub>H</sub>” and “V<sub>L</sub>”, respectively. These domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

[0050] The term “*variable*” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies, such as anti-C1s antibodies of the present disclosure. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the variability is not evenly distributed across the entire span of the variable domains. Instead, it is concentrated in three segments called hypervariable regions (HVRs) both in the light-chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework regions (FR). The variable domains of native heavy and light chains each comprise four FR regions, largely adopting a beta-sheet configuration, connected by three HVRs, which form loops connecting, and in some cases forming part of, the beta-sheet structure. The HVRs in each chain are held together in close proximity by the FR regions and, with the HVRs from the other chain, contribute to the formation of the antigen binding site of antibodies (*see* Kabat et al., *Sequences of Immunological Interest*, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant domains are not involved directly in the binding of antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent-cellular toxicity.

[0051] The term “*monoclonal antibody*” as used herein refers to an antibody, such as an anti-C1s antibody of the present disclosure, obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for

possible naturally occurring mutations and/or post-translation modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. In contrast to polyclonal antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by a variety of techniques, including, for example, the hybridoma method (*e.g.*, Kohler and Milstein., *Nature*, 256:495-97 (1975); Hongo et al., *Hybridoma*, 14 (3):253-260 (1995), Harlow et al., *Antibodies: A Laboratory Manual*, (Cold Spring Harbor Laboratory Press, 2d ed. 1988); Hammerling et al., in: *Monoclonal Antibodies and T-Cell Hybridomas* 563-681 (Elsevier, N.Y., 1981)), recombinant DNA methods (see, *e.g.*, U.S. Patent No. 4,816,567), phage-display technologies (see, *e.g.*, Clackson et al., *Nature*, 352:624-628 (1991); Marks et al., *J. Mol. Biol.* 222:581-597 (1992); Sidhu et al., *J. Mol. Biol.* 338(2): 299-310 (2004); Lee et al., *J. Mol. Biol.* 340(5):1073-1093 (2004); Fellouse, *Proc. Nat'l Acad. Sci. USA* 101(34):12467-472 (2004); and Lee et al., *J. Immunol. Methods* 284(1-2):119-132 (2004), and technologies for producing human or human-like antibodies in animals that have parts or all of the human immunoglobulin loci or genes encoding human immunoglobulin sequences (see, *e.g.*, WO 1998/24893; WO 1996/34096; WO 1996/33735; WO 1991/10741; Jakobovits et al., *Proc. Nat'l Acad. Sci. USA* 90:2551 (1993); Jakobovits et al., *Nature* 362:255-258 (1993); Bruggemann et al., *Year in Immunol.* 7:33 (1993); U.S. Patent Nos. 5,545,807; 5,545,806; 5,569,825; 5,625,126; 5,633,425; and 5,661,016; Marks et al., *Bio/Technology* 10:779-783 (1992); Lonberg et al., *Nature* 368:856-859 (1994); Morrison, *Nature* 368:812-813 (1994); Fishwild et al., *Nature Biotechnol.* 14:845-851 (1996); Neuberger, *Nature Biotechnol.* 14:826 (1996); and Lonberg and Huszar, *Intern. Rev. Immunol.* 13:65-93 (1995).

**[0052]** The terms “full-length antibody,” “intact antibody” or “whole antibody” are used interchangeably to refer to an antibody, such as and anti-C1s antibody of the present disclosure, in its substantially intact form, as opposed to an antibody fragment. Specifically whole antibodies include those with heavy and light chains including an Fc region. The constant

domains may be native sequence constant domains (e.g., human native sequence constant domains) or amino acid sequence variants thereof. In some cases, the intact antibody may have one or more effector functions.

[0053] An “*antibody fragment*” comprises a portion of an intact antibody, preferably the antigen binding and/or the variable region of the intact antibody. Examples of antibody fragments include Fab, Fab', F(ab')<sub>2</sub> and Fv fragments; diabodies; linear antibodies (see U.S. Patent 5,641,870, Example 2; Zapata et al., *Protein Eng.* 8(10):1057-1062 (1995)); single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0054] Papain digestion of antibodies, such as anti-C1s antibodies of the present disclosure, produces two identical antigen-binding fragments, called “*Fab*” fragments, and a residual “*Fc*” fragment, a designation reflecting the ability to crystallize readily. The Fab fragment consists of an entire L chain along with the variable region domain of the H chain (V<sub>H</sub>), and the first constant domain of one heavy chain (C<sub>H1</sub>). Each Fab fragment is monovalent with respect to antigen binding, i.e., it has a single antigen-binding site. Pepsin treatment of an antibody yields a single large F(ab')<sub>2</sub> fragment which roughly corresponds to two disulfide linked Fab fragments having different antigen-binding activity and is still capable of cross-linking antigen. Fab' fragments differ from Fab fragments by having a few additional residues at the carboxy terminus of the C<sub>H1</sub> domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. F(ab')<sub>2</sub> antibody fragments originally were produced as pairs of Fab' fragments which have hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

[0055] The Fc fragment comprises the carboxy-terminal portions of both H chains held together by disulfides. The effector functions of antibodies are determined by sequences in the Fc region, the region which is also recognized by Fc receptors (FcR) found on certain types of cells.

[0056] “*Fv*” is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This fragment consists of a dimer of one heavy- and one light-chain variable region domain in tight, non-covalent association. From the folding of these two domains emanate six hypervariable loops (3 loops each from the H and L chain) that contribute the amino acid residues for antigen binding and confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three HVRs

specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

[0057] “Single-chain Fv” also abbreviated as “sFv” or “scFv” are antibody fragments that comprise the V<sub>H</sub> and V<sub>L</sub> antibody domains connected into a single polypeptide chain.

5 Preferably, the sFv polypeptide further comprises a polypeptide linker between the V<sub>H</sub> and V<sub>L</sub> domains which enables the sFv to form the desired structure for antigen binding. For a review of the sFv, see Plückthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds., Springer-Verlag, New York, pp. 269-315 (1994).

[0058] “Functional fragments” of antibodies, such as anti-C1s antibodies of the present  
10 disclosure, comprise a portion of an intact antibody, generally including the antigen binding or variable region of the intact antibody or the F region of an antibody which retains or has modified FcR binding capability. Examples of antibody fragments include linear antibody, single-chain antibody molecules and multispecific antibodies formed from antibody fragments.

[0059] The term “diabodies” refers to small antibody fragments prepared by constructing sFv  
15 fragments (see preceding paragraph) with short linkers (about 5-10) residues) between the V<sub>H</sub> and V<sub>L</sub> domains such that inter-chain but not intra-chain pairing of the V domains is achieved, thereby resulting in a bivalent fragment, *i.e.*, a fragment having two antigen-binding sites. Bispecific diabodies are heterodimers of two “crossover” sFv fragments in which the V<sub>H</sub> and V<sub>L</sub> domains of the two antibodies are present on different polypeptide chains. Diabodies are  
20 described in greater detail in, for example, EP 404,097; WO 93/11161; Hollinger et al., *Proc. Nat’l Acad. Sci. USA* 90:6444-48 (1993).

[0060] As used herein, a “chimeric antibody” refers to an antibody (immunoglobulin), such as an anti-C1s antibody of the present disclosure, in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a  
25 particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., *Proc. Nat’l Acad. Sci. USA*, 81:6851-55 (1984)). Chimeric  
30 antibodies of interest herein include PRIMATIZED<sup>®</sup> antibodies wherein the antigen-binding region of the antibody is derived from an antibody produced by, *e.g.*, immunizing macaque

monkeys with an antigen of interest. As used herein, “humanized antibody” is a subset of “chimeric antibodies.”

[0061] “*Humanized*” forms of non-human (*e.g.*, murine) antibodies, such as anti-C1s antibodies of the present disclosure, are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In one embodiment, a humanized antibody is a human immunoglobulin (recipient antibody) in which residues from an HVR of the recipient are replaced by residues from an HVR of a non-human species (donor antibody) such as mouse, rat, rabbit or non-human primate having the desired specificity, affinity, and/or capacity. In some instances, FR residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues that are not found in the recipient antibody or in the donor antibody. These modifications may be made to further refine antibody performance, such as binding affinity. In general, a humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin sequence, and all or substantially all of the FR regions are those of a human immunoglobulin sequence, although the FR regions may include one or more individual FR residue substitutions that improve antibody performance, such as binding affinity, isomerization, immunogenicity, and the like. The number of these amino acid substitutions in the FR is typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, *see, e.g.*, Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). *See also*, for example, Vaswani and Hamilton, *Ann. Allergy, Asthma & Immunol.* 1:105-115 (1998); Harris, *Biochem. Soc. Transactions* 23:1035-1038 (1995); Hurle and Gross, *Curr. Op. Biotech.* 5:428-433 (1994); and U.S. Patent Nos. 6,982,321 and 7,087,409.

[0062] A “*human antibody*” is one that possesses an amino-acid sequence corresponding to that of an antibody, such as an anti-C1s antibody of the present disclosure, produced by a human and/or has been made using any of the techniques for making human antibodies as disclosed herein. This definition of a human antibody specifically excludes a humanized antibody comprising non-human antigen-binding residues. Human antibodies can be produced using various techniques known in the art, including phage-display libraries. Hoogenboom and Winter, *J. Mol. Biol.*, 227:381 (1991); Marks et al., *J. Mol. Biol.*, 222:581 (1991). Also available for the

preparation of human monoclonal antibodies are methods described in Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, p. 77 (1985); Boerner et al., *J. Immunol.*, 147(1):86-95 (1991). *See also* van Dijk and van de Winkel, *Curr. Opin. Pharmacol.* 5:368-74 (2001). Human antibodies can be prepared by administering the antigen to a transgenic animal that has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled, *e.g.*, immunized xenomice (*see, e.g.*, U.S. Patent Nos. 6,075,181 and 6,150,584 regarding XENOMOUSE™ technology). *See also*, for example, Li et al., *Proc. Nat'l Acad. Sci. USA*, 103:3557-3562 (2006) regarding human antibodies generated via a human B-cell hybridoma technology.

**[0063]** The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain, such as that of an anti-C1s antibody of the present disclosure, that are hypervariable in sequence and/or form structurally defined loops. Generally, antibodies comprise six HVRs; three in the VH (H1, H2, H3), and three in the VL (L1, L2, L3). In native antibodies, H3 and L3 display the most diversity of the six HVRs, and H3 in particular is believed to play a unique role in conferring fine specificity to antibodies. *See, e.g.*, Xu et al., *Immunity* 13:37-45 (2000); Johnson and Wu in *Methods in Molecular Biology* 248:1-25 (Lo, ed., Human Press, Totowa, NJ, 2003)). Indeed, naturally occurring camelid antibodies consisting of a heavy chain only are functional and stable in the absence of light chain. *See, e.g.*, Hamers-Casterman et al., *Nature* 363:446-448 (1993) and Sheriff et al., *Nature Struct. Biol.* 3:733-736 (1996).

**[0064]** A number of HVR delineations are in use and are encompassed herein. The HVRs that are Kabat complementarity-determining regions (CDRs) are based on sequence variability and are the most commonly used (Kabat et al., *supra*). Chothia refers instead to the location of the structural loops (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)). The AbM HVRs represent a compromise between the Kabat CDRs and Chothia structural loops, and are used by Oxford Molecular's AbM antibody-modeling software. The “contact” HVRs are based on an analysis of the available complex crystal structures. The residues from each of these HVRs are noted below.

| Loop | Kabat   | AbM     | Chothia | Contact |
|------|---------|---------|---------|---------|
| L1   | L24-L34 | L24-L34 | L26-L32 | L30-L36 |
| L2   | L50-L56 | L50-L56 | L50-L52 | L46-L55 |
| L3   | L89-L97 | L89-L97 | L91-L96 | L89-L96 |

|    |          |          |          |                             |
|----|----------|----------|----------|-----------------------------|
| H1 | H31-H35B | H26-H35B | H26-H32  | H30-H35B (Kabat numbering)  |
| H1 | H31-H35  | H26-H35  | H26-H32  | H30-H35 (Chothia numbering) |
| H2 | H50-H65  | H50-H58  | H53-H55  | H47-H58                     |
| H3 | H95-H102 | H95-H102 | H96-H101 | H93-H101                    |

5 **[0065]** HVRs may comprise “extended HVRs” as follows: 24-36 or 24-34 (L1), 46-56 or 50-56 (L2), and 89-97 or 89-96 (L3) in the VL, and 26-35 (H1), 50-65 or 49-65 (a preferred embodiment) (H2), and 93-102, 94-102, or 95-102 (H3) in the VH. The variable-domain residues are numbered according to Kabat et al., *supra*, for each of these extended-HVR definitions.

10 **[0066]** “*Framework*” or “*FR*” residues are those variable-domain residues other than the HVR residues as herein defined.

**[0067]** The phrase “*variable-domain residue-numbering as in Kabat*” or “*amino-acid-position numbering as in Kabat*,” and variations thereof, refers to the numbering system used for heavy-chain variable domains or light-chain variable domains of the compilation of antibodies in Kabat et al., *supra*. Using this numbering system, the actual linear amino acid sequence may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or HVR of the variable domain. For example, a heavy-chain variable domain may include a single amino acid insert (residue 52a according to Kabat) after residue 52 of H2 and inserted residues (*e.g.*, residues 82a, 82b, and 82c, *etc.* according to Kabat) after heavy-chain FR residue 15 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a “standard” Kabat numbered sequence.

**[0068]** The Kabat numbering system is generally used when referring to a residue in the variable domain (approximately residues 1-107 of the light chain and residues 1-113 of the heavy chain) (*e.g.*, Kabat et al., Sequences of Immunological Interest. 5th Ed. Public Health Service, 25 National Institutes of Health, Bethesda, Md. (1991)). The “EU numbering system” or “EU index” is generally used when referring to a residue in an immunoglobulin heavy chain constant region (*e.g.*, the EU index reported in Kabat *et al.*, *supra*). The “EU index as in Kabat” refers to the residue numbering of the human IgG1 EU antibody. Unless stated otherwise herein, 30 references to residue numbers in the variable domain of antibodies means residue numbering by the Kabat numbering system. Unless stated otherwise herein, references to residue numbers in

the constant domain of antibodies means residue numbering by the EU numbering system (*e.g.*, *see* United States Patent Publication No. 2010-280227).

**[0069]** An “*acceptor human framework*” as used herein is a framework comprising the amino acid sequence of a VL or VH framework derived from a human immunoglobulin framework or a human consensus framework. An acceptor human framework “derived from” a human immunoglobulin framework or a human consensus framework may comprise the same amino acid sequence thereof, or it may contain pre-existing amino acid sequence changes. In some embodiments, the number of pre-existing amino acid changes are 10 or less, 9 or less, 8 or less, 7 or less, 6 or less, 5 or less, 4 or less, 3 or less, or 2 or less. Where pre-existing amino acid changes are present in a VH, preferable those changes occur at only three, two, or one of positions 71H, 73H and 78H; for instance, the amino acid residues at those positions may be 71A, 73T and/or 78A. In one embodiment, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

**[0070]** A “*human consensus framework*” is a framework that represents the most commonly occurring amino acid residues in a selection of human immunoglobulin VL or VH framework sequences. Generally, the selection of human immunoglobulin VL or VH sequences is from a subgroup of variable domain sequences. Generally, the subgroup of sequences is a subgroup as in Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD (1991). Examples include for the VL, the subgroup may be subgroup kappa I, kappa II, kappa III or kappa IV as in Kabat *et al.*, *supra*. Additionally, for the VH, the subgroup may be subgroup I, subgroup II, or subgroup III as in Kabat *et al.*, *supra*.

**[0071]** An “*amino-acid modification*” at a specified position, *e.g.*, of an anti-C1s antibody of the present disclosure, refers to the substitution or deletion of the specified residue, or the insertion of at least one amino acid residue adjacent the specified residue. Insertion “adjacent” to a specified residue means insertion within one to two residues thereof. The insertion may be N-terminal or C-terminal to the specified residue. The preferred amino acid modification herein is a substitution.

**[0072]** An “*affinity-matured*” antibody, such as an anti- C1s antibody of the present disclosure, is one with one or more alterations in one or more HVRs thereof that result in an improvement in the affinity of the antibody for antigen, compared to a parent antibody that does

not possess those alteration(s). In one embodiment, an affinity-matured antibody has nanomolar or even picomolar affinities for the target antigen. Affinity-matured antibodies are produced by procedures known in the art. For example, Marks et al., *Bio/Technology* 10:779-783 (1992) describes affinity maturation by VH- and VL-domain shuffling. Random mutagenesis of HVR and/or framework residues is described by, for example: Barbas et al. *Proc Nat. Acad. Sci. USA* 91:3809-3813 (1994); Schier et al. *Gene* 169:147-155 (1995); Yelton et al. *J. Immunol.* 155:1994-2004 (1995); Jackson et al., *J. Immunol.* 154(7):3310-9 (1995); and Hawkins et al., *J. Mol. Biol.* 226:889-896 (1992).

**[0073]** As use herein, the term “specifically recognizes” or “specifically binds” refers to measurable and reproducible interactions such as attraction or binding between a target and an antibody, such as an anti-C1s antibody of the present disclosure, that is determinative of the presence of the target in the presence of a heterogeneous population of molecules including biological molecules. For example, an antibody, such as an anti-C1s antibody of the present disclosure, that specifically or preferentially binds to a target or an epitope is an antibody that binds this target or epitope with greater affinity, avidity, more readily, and/or with greater duration than it binds to other targets or other epitopes of the target. It is also understood by reading this definition that, for example, an antibody (or a moiety) that specifically or preferentially binds to a first target may or may not specifically or preferentially bind to a second target. As such, “specific binding” or “preferential binding” does not necessarily require (although it can include) exclusive binding. An antibody that specifically binds to a target may have an association constant of at least about  $10^3 \text{ M}^{-1}$  or  $10^4 \text{ M}^{-1}$ , sometimes about  $10^5 \text{ M}^{-1}$  or  $10^6 \text{ M}^{-1}$ , in other instances about  $10^6 \text{ M}^{-1}$  or  $10^7 \text{ M}^{-1}$ , about  $10^8 \text{ M}^{-1}$  to  $10^9 \text{ M}^{-1}$ , or about  $10^{10} \text{ M}^{-1}$  to  $10^{11} \text{ M}^{-1}$  or higher. A variety of immunoassay formats can be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See, e.g., Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York, for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity.

**[0074]** As used herein, an “interaction” between a complement protein, such as complement factor C1s, and a second protein encompasses, without limitation, protein-protein interaction, a physical interaction, a chemical interaction, binding, covalent binding, and ionic binding. As used herein, an antibody “inhibits interaction” between two proteins when the antibody disrupts,

reduces, or completely eliminates an interaction between the two proteins. An antibody of the present disclosure, or fragment thereof, “inhibits interaction” between two proteins when the antibody or fragment thereof binds to one of the two proteins.

[0075] A “*blocking*” antibody, an “*antagonist*” antibody, an “*inhibitory*” antibody, or a “*neutralizing*” antibody is an antibody, such as an anti-C1s antibody of the present disclosure that inhibits or reduces one or more biological activities of the antigen it binds, such as interactions with one or more proteins. In some embodiments, blocking antibodies, antagonist antibodies, inhibitory antibodies, or “*neutralizing*” antibodies substantially or completely inhibit one or more biological activities or interactions of the antigen.

[0076] Antibody “*effector functions*” refer to those biological activities attributable to the Fc region (a native sequence Fc region or amino acid sequence variant Fc region) of an antibody, and vary with the antibody isotype.

[0077] The term “*Fc region*” herein is used to define a C-terminal region of an immunoglobulin heavy chain, including native-sequence Fc regions and variant Fc regions.

Although the boundaries of the Fc region of an immunoglobulin heavy chain might vary, the human IgG heavy-chain Fc region is usually defined to stretch from an amino acid residue at position Cys226, or from Pro230, to the carboxyl-terminus thereof. The C-terminal lysine (residue 447 according to the EU numbering system) of the Fc region may be removed, for example, during production or purification of the antibody, or by recombinantly engineering the nucleic acid encoding a heavy chain of the antibody. Accordingly, a composition of intact antibodies may comprise antibody populations with all K447 residues removed, antibody populations with no K447 residues removed, and antibody populations having a mixture of antibodies with and without the K447 residue. Suitable native-sequence Fc regions for use in the antibodies of the invention include human IgG1, IgG2, IgG3 and IgG4.

[0078] A “*native sequence Fc region*” comprises an amino acid sequence identical to the amino acid sequence of an Fc region found in nature. Native sequence human Fc regions include a native sequence human IgG1 Fc region (non-A and A allotypes); native sequence human IgG2 Fc region; native sequence human IgG3 Fc region; and native sequence human IgG4 Fc region as well as naturally occurring variants thereof.

[0079] A “*variant Fc region*” comprises an amino acid sequence which differs from that of a native sequence Fc region by virtue of at least one amino acid modification, preferably one or more amino acid substitution(s). Preferably, the variant Fc region has at least one amino acid

substitution compared to a native sequence Fc region or to the Fc region of a parent polypeptide, *e.g.* from about one to about ten amino acid substitutions, and preferably from about one to about five amino acid substitutions in a native sequence Fc region or in the Fc region of the parent polypeptide. The variant Fc region herein will preferably possess at least about 80% homology with a native sequence Fc region and/or with an Fc region of a parent polypeptide, and most preferably at least about 90% homology therewith, more preferably at least about 95% homology therewith.

**[0080]** “*Fc receptor*” or “*FcR*” describes a receptor that binds to the Fc region of an antibody. The preferred FcR is a native sequence human FcR. Moreover, a preferred FcR is one which binds an IgG antibody (a gamma receptor) and includes receptors of the FcγRI, FcγRII, and FcγRIII subclasses, including allelic variants and alternatively spliced forms of these receptors, FcγRII receptors include FcγRIIA (an “activating receptor”) and FcγRIIB (an “inhibiting receptor”), which have similar amino acid sequences that differ primarily in the cytoplasmic domains thereof. Activating receptor FcγRIIA contains an immunoreceptor tyrosine-based activation motif (“ITAM”) in its cytoplasmic domain. Inhibiting receptor FcγRIIB contains an immunoreceptor tyrosine-based inhibition motif (“ITIM”) in its cytoplasmic domain. (*see, e.g., M. Daëron, Annu. Rev. Immunol.* 15:203-234 (1997)). FcRs are reviewed in Ravetch and Kinet, *Annu. Rev. Immunol.* 9:457-92 (1991); Capel et al., *Immunomethods* 4:25-34 (1994); and de Haas et al., *J. Lab. Clin. Med.* 126: 330-41 (1995). Other FcRs, including those to be identified in the future, are encompassed by the term “FcR” herein. FcRs can also increase the serum half-life of antibodies.

**[0081]** Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding polypeptides can be assayed, *e.g.*, in transgenic mice or transfected human cell lines expressing human FcRn, or in primates to which the polypeptides having a variant Fc region are administered. WO 2004/42072 (Presta) describes antibody variants with improved or diminished binding to FcRs. *See also, e.g., Shields et al., J. Biol. Chem.* 9(2):6591-6604 (2001).

**[0082]** The term “ $k_{on}$ ”, as used herein, is intended to refer to the rate constant for association of an antibody to an antigen.

**[0083]** The term “ $k_{off}$ ”, as used herein, is intended to refer to the rate constant for dissociation of an antibody from the antibody/antigen complex.

**[0084]** The term “ $K_D$ ”, as used herein, is intended to refer to the equilibrium dissociation constant of an antibody-antigen interaction.

[0085] As used herein, “percent (%) amino acid sequence identity” and “homology” with respect to a peptide, polypeptide or antibody sequence refers to the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the specific peptide or polypeptide sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or MEGALIGN<sup>TM</sup> (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms known in the art needed to achieve maximal alignment over the full length of the sequences being compared.

[0086] An “isolated” molecule or cell is a molecule or a cell that is identified and separated from at least one contaminant molecule or cell with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated molecule or cell is free of association with all components associated with the production environment. The isolated molecule or cell is in a form other than in the form or setting in which it is found in nature. Isolated molecules therefore are distinguished from molecules existing naturally in cells; isolated cells are distinguished from cells existing naturally in tissues, organs, or individuals. In some embodiments, the isolated molecule is an anti-C1s antibody of the present disclosure. In other embodiments, the isolated cell is a host cell or hybridoma cell producing an anti-C1s antibody of the present disclosure.

[0087] An “isolated” nucleic acid molecule encoding an antibody, such as an anti-C1s antibody of the present disclosure, is a nucleic acid molecule that is identified and separated from at least one contaminant nucleic acid molecule with which it is ordinarily associated in the environment in which it was produced. Preferably, the isolated nucleic acid is free of association with all components associated with the production environment. The isolated nucleic acid molecules encoding the polypeptides and antibodies herein is in a form other than in the form or setting in which it is found in nature. Isolated nucleic acid molecules therefore are distinguished from nucleic acid encoding the polypeptides and antibodies herein existing naturally in cells.

[0088] The term “vector,” as used herein, is intended to refer to a nucleic acid molecule capable of transporting another nucleic acid to which it has been linked. One type of vector is a “plasmid,” which refers to a circular double stranded DNA into which additional DNA segments

may be ligated. Another type of vector is a phage vector. Another type of vector is a viral vector, wherein additional DNA segments may be ligated into the viral genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (*e.g.*, bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors  
5 (*e.g.*, non-episomal mammalian vectors) can be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as “recombinant expression vectors,” or simply, “expression vectors.” In general, expression vectors of utility in recombinant DNA techniques  
10 are often in the form of plasmids. In the present specification, “plasmid” and “vector” may be used interchangeably as the plasmid is the most commonly used form of vector.

**[0089]** “*Polynucleotide*,” or “*nucleic acid*,” as used interchangeably herein, refer to polymers of nucleotides of any length, and include DNA and RNA. The nucleotides can be deoxyribonucleotides, ribonucleotides, modified nucleotides or bases, and/or their analogs, or  
15 any substrate that can be incorporated into a polymer by DNA or RNA polymerase or by a synthetic reaction. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and their analogs. If present, modification to the nucleotide structure may be imparted before or after assembly of the polymer. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may comprise modification(s)  
20 made after synthesis, such as conjugation to a label. Other types of modifications include, for example, “caps,” substitution of one or more of the naturally occurring nucleotides with an analog, internucleotide modifications such as, for example, those with uncharged linkages (*e.g.*, methyl phosphonates, phosphotriesters, phosphoramidates, carbamates, *etc.*) and with charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), those containing pendant moieties,  
25 such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, *etc.*), those with intercalators (*e.g.*, acridine, psoralen, *etc.*), those containing chelators (*e.g.*, metals, radioactive metals, boron, oxidative metals, *etc.*), those containing alkylators, those with modified linkages (*e.g.*, alpha anomeric nucleic acids, *etc.*), as well as unmodified forms of the polynucleotides(s). Further, any of the hydroxyl groups ordinarily present in the sugars may be  
30 replaced, for example, by phosphonate groups, phosphate groups, protected by standard protecting groups, or activated to prepare additional linkages to additional nucleotides, or may be conjugated to solid or semi-solid supports. The 5’ and 3’ terminal OH can be phosphorylated or

substituted with amines or organic capping group moieties of from 1 to 20 carbon atoms. Other hydroxyls may also be derivatized to standard protecting groups. Polynucleotides can also contain analogous forms of ribose or deoxyribose sugars that are generally known in the art, including, for example, 2'-O-methyl-, 2'-O-allyl-, 2'-fluoro- or 2'-azido-ribose, carbocyclic sugar analogs,  $\alpha$ -anomeric sugars, epimeric sugars such as arabinose, xyloses or lyxoses, pyranose sugars, furanose sugars, sedoheptuloses, acyclic analogs, and basic nucleoside analogs such as methyl riboside. One or more phosphodiester linkages may be replaced by alternative linking groups. These alternative linking groups include, but are not limited to, embodiments wherein phosphate is replaced by P(O)S ("thioate"), P(S)S ("dithioate"), (O)NR<sub>2</sub> ("amidate"), P(O)R, P(O)OR', CO, or CH<sub>2</sub> ("formacetal"), in which each R or R' is independently H or substituted or unsubstituted alkyl (1-20 C) optionally containing an ether (-O-) linkage, aryl, alkenyl, cycloalkyl, cycloalkenyl or araldyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

**[0090]** A "*host cell*" includes an individual cell or cell culture that can be or has been a recipient for vector(s) for incorporation of polynucleotide inserts. Host cells include progeny of a single host cell, and the progeny may not necessarily be completely identical (in morphology or in genomic DNA complement) to the original parent cell due to natural, accidental, or deliberate mutation. A host cell includes cells transfected *in vivo* with a polynucleotide(s) of this invention.

**[0091]** "*Carriers*" as used herein include pharmaceutically acceptable carriers, excipients, or stabilizers that are nontoxic to the cell or mammal being exposed thereto at the dosages and concentrations employed. Often the physiologically acceptable carrier is an aqueous pH buffered solution. Examples of physiologically acceptable carriers include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptide; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as TWEEN<sup>TM</sup>, polyethylene glycol (PEG), and PLURONICS<sup>TM</sup>.

**[0092]** The term "*about*" as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to "*about*" a value or

parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*.

[0093] As used herein and in the appended claims, the singular forms “a,” “an,” and “the” include plural reference unless the context clearly indicates otherwise. For example, reference to an “antibody” is a reference to from one to many antibodies, such as molar amounts, and includes equivalents thereof known to those skilled in the art, and so forth.

[0094] It is understood that aspect and embodiments of the invention described herein include “comprising,” “consisting,” and “consisting essentially of” aspects and embodiments.

### **Overview**

[0095] The present disclosure provides neutralizing anti-C1s antibodies and uses therefore. In some embodiments, the present disclosure provides neutralizing monoclonal murine anti-C1s antibodies 5A1 and 5C12, which are produced by hybridoma cell lines deposited with ATCC on 5/15/2013 and having ATCC Accession Numbers PTA-120351 and PTA-120352, and antibodies derived from anti-C1s antibodies 5A1 and 5C12. Uses for neutralizing anti-C1s antibodies include, without limitation, the detection of complement factor C1s. Additional non-limiting uses include the inhibition of the classical pathway of complement activation, *e.g.*, in cases where the classical complement pathway is activated by autoantibodies, such as NMO-specific autoantibodies. Further non-limiting uses for neutralizing anti-C1s antibodies include the diagnosis and treatment of disorders associated with increased activation of the classical complement pathway, in particular autoimmune disorders and neurodegenerative disorders, including neurodegenerative disorders associated with synapse loss.

[0096] Accordingly, in one aspect, the present disclosure provides an anti-complement factor 1 (CF1) monoclonal antibody which binds to and neutralizes a biological activity of C1s. In some embodiments, the anti-C1s antibodies of this disclosure also bind to the C1s proenzyme.

The neutralizing anti-C1s antibodies may neutralize, without limitation, one or more biological activities of C1s. Such biological activities include, without limitation, C1s binding to C1q or C1s binding to C1r, as well as C1s binding to C2 or C4. Other non-limiting biological activities of C1s include the proteolytic enzyme activity of C1s or the conversion of the C1s proenzyme (C1s-pro) to an active C1s protease. Other biological activities include the cleavage of C4 and/or C2. Other non-limiting biological activities of C1s include the activation of the classical complement activation pathway, the activation of antibody and complement dependent

cytotoxicity, and C1F hemolysis. In some embodiments, the anti-C1s antibodies of this disclosure may bind to C1 complex.

[0097] In another aspect, the present disclosure provides an isolated nucleic acid molecule encoding an antibody of this disclosure.

5 [0098] The present disclosure also provides isolated host cells containing a nucleic acid molecule that encodes an antibody of this disclosure. In some embodiments, isolated host cell lines are provided that can produce the neutralizing monoclonal murine antibodies 5A1 and 5C12. Such isolated host cell lines were deposited with ATCC on 5/15/2013 and have ATCC Accession Numbers PTA-120351 and PTA-120352.

10 [0099] Additionally, pharmaceutical compositions are provided containing C1s neutralizing antibodies of this disclosure in combination with pharmaceutically acceptable carriers. The present disclosure also provides a kit containing an anti-C1s antibody for use in any of the methods described herein.

[0100] The present disclosure further provides methods of using the C1s neutralizing  
15 antibodies of this disclosure to treat or prevent an autoimmune or neurodegenerative disease in a subject in need of such treatment, to detect synapses in an individual having an autoimmune or neurodegenerative disease, and to detect synapses in a biological sample. The present disclosure also provides diagnostic kits containing the C1s neutralizing antibodies of this disclosure.

### **Complement Proteins**

20 [0101] The antibodies of this disclosure specifically recognize complement factor C1s and/or C1s in the C1 complex of the classical complement activation pathway. The recognized complement factor may be derived, without limitation, from any organism having a complement system, including any mammalian organism such as human, mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep, goat, or pig.

25 [0102] As used herein "*C1 complex*" refers to a protein complex that may include, without limitation, one C1q protein, two C1r proteins, and two C1s proteins (*e.g.*, C1qr<sup>2</sup>s<sup>2</sup>).

[0103] As used herein "*complement factor C1s*" refers to both wild type sequences and naturally occurring variant sequences.

[0104] A non-limiting example of a complement factor C1s recognized by antibodies of this  
30 invention is human C1s (Accession No. Protein Data Base: NP\_958850.1; GenBank No.: NM\_201442.2):

>gil41393602|reflNP\_958850.1| complement C1s subcomponent precursor [Homo sapiens]  
 MWCIVLFSLLAWVYAEPTMYGEILSPNYPQAYPSEVEKSWDIEVPEGYGIHLYFTHLDIE  
 LSENCAYDSVQIISGDTEEGRLCGQRSSNNPHSPIVEEFQVPYNKLQVIFKSDFSNEERFTG  
 FAAYYVATDINECTDFVDVPCSHFCNNFIGGYFCSCPPEYFLHDDMKNCGVNCSGDVFT  
 5 ALIGEIASPNYPKYPENSRCYQIRLEKGFQVVVTLRREDFDVEAADSAGNCLDSL VFV  
 AGDRQFGPYCGHGFPGPLNIETKSNALDIIFQTDLTGQKKGWKLRYHGDPMPCPKEDTP  
 NSVWEPAAKAKYVFRDVVQITCLDGFEVVEGRVGATSFYSTCQSNGKWSNSKLKCQPVD  
 CGIPESIEGKVEDPESTLFGSVIRYTCEEPYYMENGGGGEYHCAGNGSWVNEVLGPE  
 LPKCVPVCGVPREPFEKQRIIGGSDADIKNFPWQVFFDNPWAGGALINEYWVLTAHV  
 10 VEGNREPTMYVGSTSVQTSRLAKSKMLTPEHVFHPGWKLLEVPEGRTNFDNDIALVRL  
 KDPVKMGPTVSPICLPGTSSDYNLMDGDLGLISGWGRTEKRDRAVRLKAARLPVAPLRK  
 CEVKVEKPTADAEAYVFTPNMICAGGEKGMDSCKGDSGGAFVQDPNDKTKFYAAG  
 LVSWGPPCGTYGLYTRVKNYVDWIMKTMQENSTPRED (SEQ ID NO: 1)

[0105] Accordingly, an anti-C1s antibody of the present disclosure may bind to human C1s  
 15 or a homolog thereof, such as mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep,  
 goat, or pig C1s.

### **Anti-C1s Antibodies**

[0106] The antibodies of this disclosure recognize and bind to complement factor C1s and/or  
 C1s in the C1 complex of the classical complement activation pathway.

20 [0107] In some embodiments, the antibodies neutralize an activity of complement factor C1s.  
 In some embodiments, the antibodies inhibit the interaction between complement factor C1s and  
 other complement factors, such as C1q or C1r, or complement protease substrates, such as C4  
 and C2. In some embodiments, the antibodies inhibit the catalytic activity of the serine protease  
 C1s or inhibit the processing of a serine protease pro-form to an active protease. In some  
 25 embodiments the antibodies inhibit the classical pathway. In certain embodiments the antibodies  
 further inhibit the alternative pathway. In some embodiments, the antibodies inhibit  
 autoantibody- and complement-dependent cytotoxicity (CDC). In some embodiments, the  
 antibodies inhibit complement-dependent cell-mediated cytotoxicity (CDCC).

[0108] The functional properties of the antibodies of this invention, such as dissociation  
 30 constants for antigens, inhibition of protein-protein interactions (*e.g.*, C1s-C1q interactions),  
 inhibition of autoantibody-dependent and complement-dependent cytotoxicity (CDC), inhibition

of complement-dependent cell-mediated cytotoxicity (CDCC), or lesion formation, may, without limitation, be measured in *in vitro*, *ex vivo*, or *in vivo* experiments.

**[0109]** The dissociation constants ( $K_D$ ) of the anti-C1s antibodies for C1s may be less than 100nM, less than 90 nM, less than 80 nM, less than 70 nM, less than 60 nM, less than 50 nM, less than 40 nM, less than 30 nM, less than 20 nM, less than 10 nM, less than 9 nM, less than 8 nM, less than 7 nM, less than 6 nM, less than 5 nM, less than 4 nM, less than 3 nM, less than 2 nM, less than 1 nM, less than 0.5 nM, less than 0.1 nM, less than 0.05 nM, less than 0.01 nM, or less than 0.005 nM. Preferably, dissociation constants are less than 20 nM. Antibody dissociation constants for antigens other than C1s may be at least 5-fold, at least 10-fold, at least 100-fold, at least 1,000-fold, at least 10,000-fold, or at least 100,000-fold higher than the dissociation constants for their respective antigens. For example, the dissociation constant of a C1s antibody of this disclosure may be at least 1,000-fold higher for C1q than for C1s. Dissociation constants may be determined through any analytical technique, including any biochemical or biophysical technique such as surface plasmon resonance (SPR), isothermal titration calorimetry (ITC), differential scanning calorimetry (DSC), circular dichroism (CD), stopped-flow analysis, and colorimetric or fluorescent protein melting analyses. Dissociation constants ( $K_D$ ) of the anti-C1s antibodies for their respective antigens may be determined, *e.g.*, using full-length antibodies or antibody fragments, such as Fab fragments. The antibodies of this disclosure may bind to C1s antigens derived from any organism having a complement system, including any mammalian organism such as human, mouse, rat, rabbit, monkey, dog, cat, cow, horse, camel, sheep, goat, or pig. In preferred embodiments, the antibodies of this disclosure bind to epitopes comprising amino acid residues on human C1s.

**[0110]** In some embodiments, provided herein are anti-C1s antibodies that compete with antibody 5A1, 5C12, or an anti-C1s antibody described herein for binding to C1s. Competition assays can be used to determine whether two antibodies bind the same epitope by recognizing identical or sterically overlapping epitopes or one antibody competitively inhibits binding of another antibody to the antigen. These assays are known in the art. Typically, antigen or antigen expressing cells is immobilized on a multi-well plate and the ability of unlabeled antibodies to block the binding of labeled antibodies is measured. Common labels for such competition assays are radioactive labels or enzyme labels.

**[0111]** Competitive antibodies encompassed herein are antibodies that inhibit (*i.e.*, prevent or interfere with in comparison to a control) or reduce 5A1, 5C12, or an anti-C1s antibody

described herein binding to C1s by at least 50%, 60%, 70%, and 80% in order of increasing preference (even more preferably, at least 90% and, most preferably, at least 95%) at 1  $\mu$ M or less with 5A1, 5C12, or an anti-C1s antibody described herein at or below its  $K_D$ . Competition between binding members may be readily assayed *in vitro* for example using ELISA and/or by monitoring the interaction of the antibodies with C1s in solution. The exact means for conducting the analysis is not critical. C1s may be immobilized to a 96-well plate or may be placed in a homogenous solution. In specific embodiments, the ability of unlabeled candidate antibody or antibodies to block the binding of labeled 5A1 or 5C12 can be measured using radioactive, enzyme, or other labels. In the reverse assay, the ability of unlabeled antibodies to interfere with the interaction of labeled 5A1 or 5C12 with C1s wherein said 5A1 or 5C12 and C1s are already bound is determined. The readout is through measurement of bound label. C1s and the candidate antibody or antibodies may be added in any order or at the same time.

**[0112]** In some preferred embodiments, the anti-C1s antibody is murine anti-human C1s monoclonal antibody 5A1, which is produced by a hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120351. In some embodiments, the anti-C1s antibody is an isolated antibody which binds essentially the same C1s epitope as 5A1. In some embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody 5A1 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120351, or progeny thereof. In some embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody 5A1 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120351, or progeny thereof. In some embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains and the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody 5A1 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120351, or progeny thereof.

**[0113]** In some preferred embodiments, the anti-C1s antibody is murine anti-human C1s monoclonal antibody 5C12, which is produced by a hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120352. In some embodiments, the anti-C1s antibody is an isolated antibody which binds essentially the same C1s epitope as 5C12. In some

embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains of monoclonal antibody 5C12 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120352, or progeny thereof. In some embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody 5C12 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120352, or progeny thereof. In some embodiments, the anti-C1s antibody is an isolated antibody comprising the HVR-L1, HVR-L2, and HVR-L3 of the light chain variable domains and the HVR-H1, HVR-H2, and HVR-H3 of the heavy chain variable domains of monoclonal antibody 5A1 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 having ATCC Accession Number PTA-120352, or progeny thereof.

**[0114]** In some embodiments, the anti-C1s antibody inhibits the interaction between C1s and C1q. In some embodiments, the anti-C1s antibody inhibits the interaction between C1s and C1r.

In some embodiments the anti-C1s antibody inhibits the interaction between C1s and C1q and between C1s and C1r. In some embodiments, the anti-C1s antibody inhibits the catalytic activity of C1s or the processing of pro-C1s to an active protease. In some embodiments, the anti-C1s antibody inhibits the interaction between C1s and its substrates such as C2 and C4. In some embodiments, the anti-C1s antibody binds to C1s respective interactions, at a stoichiometry of less than 2.5:1; 2.0:1; 1.5:1; or 1.0:1. In some embodiments, the anti-C1s antibody binds to C1s with a stoichiometry of less than 20:1; less than 19.5:1; less than 19:1; less than 18.5:1; less than 18:1; less than 17.5:1; less than 17:1; less than 16.5:1; less than 16:1; less than 15.5:1; less than 15:1; less than 14.5:1; less than 14:1; less than 13.5:1; less than 13:1; less than 12.5:1; less than 12:1; less than 11.5:1; less than 11:1; less than 10.5:1; less than 10:1; less than 9.5:1; less than 9:1; less than 8.5:1; less than 8:1; less than 7.5:1; less than 7:1; less than 6.5:1; less than 6:1; less than 5.5:1; less than 5:1; less than 4.5:1; less than 4:1; less than 3.5:1; less than 3:1; less than 2.5:1; less than 2.0:1; less than 1.5:1; or less than 1.0:1. In certain embodiments, the anti-C1s antibody binds C1s with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than 1.0:1. In certain embodiments, the anti-C1s antibody binds C1s with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than 1.0:1. In certain embodiments, the anti-C1s antibody binds C1s with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than 1.0:1. In preferred embodiments, the C1s antibody inhibits an interaction, such as the C1s-C4 interaction or C1s-C2

interaction, at approximately equimolar concentrations of C1s and the anti-C1s antibody. In some embodiments the anti-C1s antibody inhibits activation of C1s, C1r, or of both C1s and C1r.

**[0115]** Where antibodies of this disclosure inhibit the interaction between two or more complement factors, such as the interaction of C1q and C1s, or the interaction between factor C1r and C1s, the interaction occurring in the presence of the antibody is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% compared to the interaction occurring in the absence of the antibody, but otherwise identical conditions. In certain embodiments, the interaction occurring in the presence of the antibody is reduced by an amount that ranges from at least 30% to at least 99% compared to the interaction occurring in the absence of the antibody, but otherwise identical conditions. Where antibodies of this disclosure inhibit activation of C1s, the serine protease activity of C1s in the presence of the antibody is reduced by at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% compared to the serine protease activity in the absence of the antibody. In certain embodiments, the serine protease activity of C1s in the presence of the antibody is reduced by an amount that ranges from at least 30% to at least 99% compared to the serine protease activity in the absence of the antibody.

**[0116]** In some embodiments, the antibodies of this disclosure inhibit C4-cleavage by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure are absent. In certain embodiments, the antibodies of this disclosure inhibit C4-cleavage by an amount that ranges from at least 30% to at least 99% relative to a control wherein the antibodies of this disclosure are absent. Methods for measuring C4-cleavage are well known in the art (*see also* Example 3 for possible methods). The EC<sub>50</sub> values for antibodies of this disclosure with respect C4-cleavage may be less than 3 µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µg/ml (*see also* Example 4). Preferably, the antibodies of this disclosure inhibit C4-cleavage at approximately equimolar concentrations of C1s and the respective anti-C1s antibody (*see also* Example 4).

**[0117]** In some embodiments, the antibodies of this disclosure inhibit C2-cleavage by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure

are absent. In certain embodiments, the antibodies of this disclosure inhibit C2-cleavage by an amount that ranges from at least 30% to at least 99% relative to a control wherein the antibodies of this disclosure are absent. Methods for measuring C2-cleavage are well known in the art. The EC<sub>50</sub> values for antibodies of this disclosure with respect C2-cleavage may be less than 3 µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µg/ml. Preferably, the antibodies of this disclosure inhibit C2-cleavage at approximately equimolar concentrations of C1s and the respective anti-C1s antibody.

**[0118]** In some embodiments, the antibodies of this disclosure inhibit autoantibody-

dependent and complement-dependent cytotoxicity (CDC) by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure are absent. In certain embodiments, antibodies of this disclosure inhibit CDC by an amount that ranges from at least 30% to at least 99%. The EC<sub>50</sub> values for antibodies of this disclosure with respect to inhibition of

autoantibody-dependent and complement-dependent cytotoxicity may be less than 3µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µg/ml.

**[0119]** In some embodiments, the antibodies of this disclosure inhibit complement-dependent

cell-mediated cytotoxicity (CDCC) by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure are absent. In certain embodiments, antibodies of this disclosure inhibit CDCC by an amount that ranges from at least 30% to at least 99%. Methods for

measuring CDCC are well known in the art. The EC<sub>50</sub> values for antibodies of this disclosure with respect CDCC inhibition may be less than 3µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µg/ml. In preferred embodiments, the antibodies of this disclosure inhibit CDCC but not antibody-dependent cellular cytotoxicity (ADCC).

**[0120]** In some embodiments, the antibodies of this disclosure inhibit C1F hemolysis by at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this

disclosure are absent (*see also* Example 3). In certain embodiments, antibodies of this disclosure inhibit C1F hemolysis by an amount that ranges from at least 30% to at least 99% relative to a control wherein the antibodies of this disclosure are absent. Methods for measuring C1F hemolysis are well known in the art (*see also* Example 3 for possible methods). The EC<sub>50</sub> values for antibodies of this disclosure with respect to C1F hemolysis may be less than less than 3 µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µg/ml (*see also* Example 3). Preferably, the antibodies of this disclosure inhibit C1F hemolysis at approximately equimolar concentrations of C1s and the respective anti-C1s antibody.

**[0121]** In some embodiments, the alternative pathway may amplify CDC initiated by C1q binding and subsequent C1s activation; in at least some of these embodiments, the antibodies of this disclosure inhibit the alternative pathway by at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or at least 99% relative to a control wherein the antibodies of this disclosure are absent. In certain embodiments, antibodies of this disclosure inhibit the alternative pathway by an amount that ranges from at least 30% to at least 99% relative to a control wherein the antibodies of this disclosure are absent.

**[0122]** In some embodiments, the antibodies of this disclosure prevent lesion formation in an *ex vivo* spinal cord slice model of NMO or in an *in vivo* mouse model of NMO. Methods for measuring lesion formation *ex vivo* or *in vivo* are well known in the art. *Ex vivo* lesion formation may be reduced at least by a relative score of 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, or 4.0. Preferably, *ex vivo* lesion formation is reduced by a relative score of at least 2.5. The EC<sub>50</sub> values for antibodies of this disclosure with respect to the prevention of *ex vivo* lesion formation may be less than 3 µg/ml; less than 2.5 µg/ml; less than 2.0 µg/ml; less than 1.5 µg/ml; less than 1.0 µg/ml; less than 0.5 µg/ml; less than 0.25 µg/ml; less than 0.1 µg/ml; or less than 0.05 µg/ml. Preferably, EC<sub>50</sub> values are less than 1.0 µM. *In vivo* lesion formation may be reduced by at least 5%, at least 10%, at least 15%, at least 20%, at least 35%, at least 40%, or at least 50% in terms of loss of staining (% of area). Staining may be assessed, without limitation, by AQP4 staining, GFAP staining, or MBP staining. Preferably, *in vivo* lesion formation is reduced by at least 10%.

**[0123]** The present disclosure provides anti-C1s antibodies. The antibodies of this disclosure may have one or more of the following characteristics. The antibodies of this disclosure may be polyclonal antibodies, monoclonal antibodies, humanized antibodies, human antibodies, antibody

fragments, bispecific and polyspecific antibodies, multivalent antibodies, or heteroconjugate antibodies. Antibody fragments of this disclosure may be functional fragments that bind the same epitope as any of the anti-C1s antibodies of this disclosure. In some embodiments, the antibody fragments of this disclosure specifically bind to and neutralize a biological activity of C1s or the C1s proenzyme. In some embodiments, the antibody fragments are miniaturized versions of the anti-C1s antibodies or antibody fragments of this disclosure that have the same epitope of the corresponding full-length antibody, but have much smaller molecule weight. Such miniaturized anti-C1s antibody fragments may have better brain penetration ability and a shorter half-life, which is advantageous for imaging and diagnostic utilities (*see e.g.*, Lütje S et al., *Bioconjug Chem.* 2014 Feb 19;25(2):335-41; Tavaré R et al., *Proc Natl Acad Sci U S A.* 2014 Jan 21;111(3):1108-13; and Wiehr S et al., *Prostate.* 2014 May;74(7):743-55). Accordingly, in some embodiments, anti-C1s antibody fragments of this disclosure have better brain penetration as compared to their corresponding full-length antibodies and/or have a shorter half-life as compared to their corresponding full-length antibodies. In some embodiments, anti-C1s antibodies of the present disclosure are bispecific antibodies recognizing a first antigen and a second antigen. In some embodiments, the first antigen is a C1s antigen. In some embodiments, the second antigen is an antigen facilitating transport across the blood-brain-barrier, including without limitation, transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an angiopep peptide, and ANG1005. The antibodies of this disclosure may further contain engineered effector functions, amino acid sequence modifications or other antibody modifications known in the art; *e.g.*, the constant region of the anti-C1s antibodies described herein may be modified to impair complement activation.

**[0124]** Additional anti-C1s antibodies, *e.g.*, antibodies that specifically bind to a C1s protein of the present disclosure, may be identified, screened, and/or characterized for their physical/chemical properties and/or biological activities by various assays known in the art.

#### *Antibody preparation*

**[0125]** Anti-C1s antibodies of the present disclosure can encompass polyclonal antibodies, monoclonal antibodies, humanized antibodies, chimeric antibodies, human antibodies, antibody fragments (*e.g.*, Fab, Fab'-SH, Fv, scFv, and F(ab')<sub>2</sub>), bispecific and polyspecific antibodies,

multivalent antibodies, heteroconjugate antibodies, library derived antibodies, antibodies having modified effector functions, fusion proteins containing an antibody portion, and any other modified configuration of the immunoglobulin molecule that includes an antigen recognition site, such as an epitope having amino acid residues of a C1s protein of the present disclosure, including glycosylation variants of antibodies, amino acid sequence variants of antibodies, and covalently modified antibodies. The anti- C1s antibodies may be human, murine, rat, or of any other origin (including chimeric or humanized antibodies).

*(1) Polyclonal antibodies*

**[0126]** Polyclonal antibodies, such as polyclonal anti-C1s antibodies, are generally raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the relevant antigen and an adjuvant. It may be useful to conjugate the relevant antigen (*e.g.*, a purified or recombinant C1s protein of the present disclosure) to a protein that is immunogenic in the species to be immunized, *e.g.*, keyhole limpet hemocyanin (KLH), serum albumin, bovine thyroglobulin, or soybean trypsin inhibitor, using a bifunctional or derivatizing agent, *e.g.*, maleimidobenzoyl sulfosuccinimide ester (conjugation through cysteine residues), N-hydroxysuccinimide (through lysine residues), glutaraldehyde, succinic anhydride,  $\text{SOCl}_2$ , or  $\text{R}^1\text{N}=\text{C}=\text{NR}$ , where R and  $\text{R}^1$  are independently lower alkyl groups. Examples of adjuvants which may be employed include Freund's complete adjuvant and MPL-TDM adjuvant (monophosphoryl Lipid A, synthetic trehalose dicorynomycolate). The immunization protocol may be selected by one skilled in the art without undue experimentation.

**[0127]** The animals are immunized against the desired antigen, immunogenic conjugates, or derivatives by combining, *e.g.*, 100  $\mu\text{g}$  (for rabbits) or 5  $\mu\text{g}$  (for mice) of the protein or conjugate with 3 volumes of Freund's complete adjuvant and injecting the solution intradermally at multiple sites. One month later, the animals are boosted with 1/5 to 1/10 the original amount of peptide or conjugate in Freund's complete adjuvant by subcutaneous injection at multiple sites. Seven to fourteen days later, the animals are bled and the serum is assayed for antibody titer. Animals are boosted until the titer plateaus. Conjugates also can be made in recombinant-cell culture as protein fusions. Also, aggregating agents such as alum are suitable to enhance the immune response.

*(2) Monoclonal antibodies*

**[0128]** Monoclonal antibodies, such as monoclonal anti-C1s antibodies, are obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising

the population are identical except for possible naturally occurring mutations and/or post-translational modifications (*e.g.*, isomerizations, amidations) that may be present in minor amounts. Thus, the modifier “monoclonal” indicates the character of the antibody as not being a mixture of discrete antibodies.

5 [0129] For example, the monoclonal anti-C1s antibodies may be made using the hybridoma method first described by Kohler et al., *Nature*, 256:495 (1975), or may be made by recombinant DNA methods (U.S. Patent No. 4,816,567).

[0130] In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable  
10 of producing antibodies that will specifically bind to the protein used for immunization (*e.g.*, a purified or recombinant C1s protein of the present disclosure). Alternatively, lymphocytes may be immunized *in vitro*. Lymphocytes then are fused with myeloma cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, *Monoclonal Antibodies: Principles and Practice*, pp.59-103 (Academic Press, 1986)).

15 [0131] The immunizing agent will typically include the antigenic protein (*e.g.*, a purified or recombinant C1s protein of the present disclosure) or a fusion variant thereof. Generally peripheral blood lymphocytes (“PBLs”) are used if cells of human origin are desired, while spleen or lymph node cells are used if non-human mammalian sources are desired. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as  
20 polyethylene glycol, to form a hybridoma cell. Goding, *Monoclonal Antibodies: Principles and Practice*, Academic Press (1986), pp. 59-103.

[0132] Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells of rodent, bovine or human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells thus prepared are seeded and grown in a suitable culture  
25 medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which are substances that prevent the growth of HGPRT-deficient-cells.

30 [0133] Preferred immortalized myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred are murine myeloma lines, such as those

derived from MOPC-21 and MPC-11 mouse tumors (available from the Salk Institute Cell Distribution Center, San Diego, California USA), as well as SP-2 cells and derivatives thereof (*e.g.*, X63-Ag8-653) (available from the American Type Culture Collection, Manassas, Virginia USA). Human myeloma and mouse-human heteromyeloma cell lines have also been described  
5 for the production of human monoclonal antibodies (Kozbor, *J. Immunol.*, 133:3001 (1984); Brodeur et al., *Monoclonal Antibody Production Techniques and Applications*, pp. 51-63 (Marcel Dekker, Inc., New York, 1987)).

**[0134]** Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen (*e.g.*, a C1s protein of the present disclosure).  
10 Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA).

**[0135]** The culture medium in which the hybridoma cells are cultured can be assayed for the presence of monoclonal antibodies directed against the desired antigen (*e.g.*, a C1s protein of the  
15 present disclosure). Preferably, the binding affinity and specificity of the monoclonal antibody can be determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked assay (ELISA). Such techniques and assays are known in the art. For example, binding affinity may be determined by the Scatchard analysis of Munson et al., *Anal. Biochem.*, 107:220 (1980).

**[0136]** After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or activity, the clones may be subcloned by limiting dilution procedures and grown  
20 by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells may be grown *in vivo* as tumors in a mammal.

**[0137]** The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose chromatography, hydroxylapatite chromatography, gel  
25 electrophoresis, dialysis, affinity chromatography, and other methods as described above.

**[0138]** Anti-C1s monoclonal antibodies may also be made by recombinant DNA methods,  
30 such as those disclosed in U.S. Patent No. 4,816,567, and as described above. DNA encoding the monoclonal antibodies is readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that specifically bind to genes encoding the heavy and light

chains of murine antibodies). The hybridoma cells serve as a preferred source of such DNA. Once isolated, the DNA may be placed into expression vectors, which are then transfected into host-cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, in order to synthesize monoclonal antibodies in such recombinant host-cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opin. Immunol.*, 5:256-262 (1993) and Plückthun, *Immunol. Rev.* 130:151-188 (1992).

[0139] In certain embodiments, anti-C1s antibodies can be isolated from antibody phage libraries generated using the techniques described in McCafferty et al., *Nature*, 348:552-554 (1990). Clackson et al., *Nature*, 352:624-628 (1991) and Marks et al., *J. Mol. Biol.*, 222:581-597 (1991) described the isolation of murine and human antibodies, respectively, from phage libraries. Subsequent publications describe the production of high affinity (nanomolar (“nM”) range) human antibodies by chain shuffling (Marks et al., *Bio/Technology*, 10:779-783 (1992)), as well as combinatorial infection and *in vivo* recombination as a strategy for constructing very large phage libraries (Waterhouse et al., *Nucl. Acids Res.*, 21:2265-2266 (1993)). Thus, these techniques are viable alternatives to traditional monoclonal antibody hybridoma techniques for isolation of monoclonal antibodies of desired specificity (*e.g.*, those that bind a C1s protein of the present disclosure).

[0140] The DNA encoding antibodies or fragments thereof may also be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (U.S. Patent No. 4,816,567; Morrison, et al., *Proc. Natl Acad. Sci. USA*, 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Typically such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody, or they are substituted for the variable domains of one antigen-combining site of an antibody to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for an antigen and another antigen-combining site having specificity for a different antigen.

[0141] The monoclonal antibodies described herein (*e.g.*, anti-C1s antibodies of the present disclosure or fragments thereof) may be monovalent, the preparation of which is well known in the art. For example, one method involves recombinant expression of immunoglobulin light chain and a modified heavy chain. The heavy chain is truncated generally at any point in the Fc region so as to prevent heavy chain crosslinking. Alternatively, the relevant cysteine residues

may be substituted with another amino acid residue or are deleted so as to prevent crosslinking. *In vitro* methods are also suitable for preparing monovalent antibodies. Digestion of antibodies to produce fragments thereof, particularly Fab fragments, can be accomplished using routine techniques known in the art.

5 [0142] Chimeric or hybrid anti-C1s antibodies also may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate.

10 (3) *Humanized antibodies*

[0143] Anti-C1s antibodies of the present disclosure or antibody fragments thereof may further include humanized or human antibodies. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fab, Fab'-SH, Fv, scFv, F(ab')<sub>2</sub> or other antigen-binding subsequences of antibodies) which  
15 contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues from a complementarity determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced  
20 by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human  
25 immunoglobulin consensus sequence. The humanized antibody optimally will also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. Jones et al., *Nature* 321: 522-525 (1986); Riechmann et al., *Nature* 332: 323-329 (1988) and Presta, *Curr. Opin. Struct. Biol.* 2: 593-596 (1992).

[0144] Methods for humanizing non-human anti-C1s antibodies are well known in the art.  
30 Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization

can be essentially performed following the method of Winter and co-workers, Jones et al., *Nature* 321:522-525 (1986); Riechmann et al., *Nature* 332:323-327 (1988); Verhoeyen et al., *Science* 239:1534-1536 (1988), or through substituting rodent CDRs or CDR sequences for the corresponding sequences of a human antibody. Accordingly, such “humanized” antibodies are  
5 chimeric antibodies (U.S. Patent No. 4,816,567), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

10 **[0145]** The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called “best-fit” method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human framework (FR) for the humanized  
15 antibody. Sims et al., *J. Immunol.*, 151:2296 (1993); Chothia et al., *J. Mol. Biol.*, 196:901 (1987). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework may be used for several different humanized antibodies. Carter et al., *Proc. Nat’l Acad. Sci. USA* 89:4285 (1992); Presta et al., *J. Immunol.* 151:2623 (1993).

20 **[0146]** Furthermore, it is important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analyzing the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly  
25 available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, *i.e.*, the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this  
30 way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen or antigens

(e.g., C1s proteins of the present disclosure), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

[0147] Various forms of the humanized anti-C1s antibody are contemplated. For example, the humanized anti-C1s antibody may be an antibody fragment, such as an Fab, which is optionally conjugated with one or more cytotoxic agent(s) in order to generate an immunoconjugate. Alternatively, the humanized anti-C1s antibody may be an intact antibody, such as an intact IgG1 antibody.

(4) *Human antibodies*

[0148] Alternatively, human anti-C1s antibodies can be generated. For example, it is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. The homozygous deletion of the antibody heavy-chain joining region ( $J_H$ ) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., *Proc. Nat'l Acad. Sci. USA*, 90:2551 (1993); Jakobovits et al., *Nature*, 362:255-258 (1993); Bruggermann et al., *Year in Immunol.*, 7:33 (1993); U.S. Patent Nos. 5,591,669 and WO 97/17852.

[0149] Alternatively, phage display technology can be used to produce human anti-C1s antibodies and antibody fragments *in vitro*, from immunoglobulin variable (V) domain gene repertoires from unimmunized donors. McCafferty et al., *Nature* 348:552-553 (1990); Hoogenboom and Winter, *J. Mol. Biol.* 227: 381 (1991). According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties. Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats, reviewed in, e.g., Johnson, Kevin S. and Chiswell, David J., *Curr. Opin Struct. Biol.* 3:564-571 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., *Nature* 352:624-628 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived from the spleens of immunized mice. A

repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581-597 (1991), or Griffith et al., EMBO J. 12:725-734 (1993). *See also* U.S. Patent. Nos. 5,565,332 and 5,573,905. Additionally, yeast display technology can be used to produce human anti-C1s antibodies and antibody fragments *in vitro* (e.g., WO 2009/036379; WO 2010/105256; WO 2012/009568; US 2009/0181855; US 2010/0056386; and Feldhaus and Siegel (2004) J. Immunological Methods 290:69-80). In other embodiments, ribosome display technology can be used to produce human anti-C1s antibodies and antibody fragments *in vitro* (e.g., Roberts and Szostak (1997) Proc Natl Acad Sci 94:12297-12302; Schaffitzel et al. (1999) J. Immunological Methods 231:119-135; Lipovsek and Plückthun (2004) J. Immunological Methods 290:51-67).

**[0150]** The techniques of Cole et al., and Boerner et al., are also available for the preparation of human anti-C1s monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, p. 77 (1985) and Boerner et al., *J. Immunol.* 147(1): 86-95 (1991).

Similarly, human anti-C1s antibodies can be made by introducing human immunoglobulin loci into transgenic animals, e.g., mice in which the endogenous immunoglobulin genes have been partially or completely inactivated. Upon challenge, human antibody production is observed, which closely resembles that seen in humans in all respects, including gene rearrangement, assembly and antibody repertoire. This approach is described, for example, in U.S. Patent Nos. 5,545,807; 5,545,806, 5,569,825, 5,625,126, 5,633,425, 5,661,016 and in the following scientific publications: Marks et al., *Bio/Technology* 10: 779-783 (1992); Lonberg et al., *Nature* 368: 856-859 (1994); Morrison, *Nature* 368: 812-13 (1994), Fishwild et al., *Nature Biotechnology* 14: 845-51 (1996), Neuberger, *Nature Biotechnology* 14: 826 (1996) and Lonberg and Huszar, *Intern. Rev. Immunol.* 13: 65-93 (1995).

**[0151]** Finally, human anti-C1s antibodies may also be generated *in vitro* by activated B-cells (see U.S. Patent Nos 5,567,610 and 5,229,275).

#### (5) Antibody fragments

**[0152]** In certain embodiments there are advantages to using anti-C1s antibody fragments, rather than whole anti-C1s antibodies. Smaller fragment sizes allow for rapid clearance.

**[0153]** Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (*see, e.g.,* Morimoto et al., *J. Biochem. Biophys. Method.* 24:107-117 (1992); and Brennan et al.,

*Science* 229:81 (1985)). However, these fragments can now be produced directly by recombinant host-cells, for example, using nucleic acids encoding anti-C1s antibodies of the present disclosure. Fab, Fv and scFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the straightforward production of large amounts of these fragments. A anti-C1s antibody fragments can also be isolated from the antibody phage libraries as discussed above. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form F(ab')<sub>2</sub> fragments (Carter et al., *Bio/Technology* 10:163-167 (1992)). According to another approach, F(ab')<sub>2</sub> fragments can be isolated directly from recombinant host-cell culture. Production of Fab and F(ab')<sub>2</sub> antibody fragments with increased *in vivo* half-lives are described in U.S. Patent No. 5,869,046. In other embodiments, the antibody of choice is a single chain Fv fragment (scFv). See WO 93/16185; U.S. Patent No. 5,571,894 and U.S. Patent No. 5,587,458. The anti-C1q, anti-C1r, or anti-C1s antibody fragment may also be a "linear antibody," *e.g.*, as described in U.S. Patent 5,641,870. Such linear antibody fragments may be monospecific or bispecific.

(6) *Bispecific and polyspecific antibodies*

**[0154]** Bispecific antibodies (BsAbs) are antibodies that have binding specificities for at least two different epitopes, including those on the same or another protein (*e.g.*, one or more C1s proteins of the present disclosure). Alternatively, one part of a BsAb can be armed to bind to the target C1s antigen, and another can be combined with an arm that binds to a second protein.

Such antibodies can be derived from full length antibodies or antibody fragments (*e.g.*, F(ab')<sub>2</sub> bispecific antibodies).

**[0155]** Methods for making bispecific antibodies are known in the art. Traditional production of full length bispecific antibodies is based on the coexpression of two immunoglobulin heavy-chain/light chain pairs, where the two chains have different specificities.

Millstein et al., *Nature*, 305:537-539 (1983). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct bispecific structure. Purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 and in Traunecker et al., *EMBO J.*, 10:3655-3659 (1991).

**[0156]** According to a different approach, antibody variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant

domain sequences. The fusion preferably is with an immunoglobulin heavy chain constant domain, comprising at least part of the hinge, C<sub>H</sub>2, and C<sub>H</sub>3 regions. It is preferred to have the first heavy-chain constant region (C<sub>H</sub>1) containing the site necessary for light chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the expression of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance.

**[0157]** In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. It was found that this asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only half of the bispecific molecules provides for an easy way of separation. This approach is disclosed in WO 94/04690. For further details of generating bispecific antibodies, see, for example, Suresh et al., *Methods in Enzymology* 121: 210 (1986).

**[0158]** According to another approach described in WO 96/27011 or U.S. Patent No. 5,731,168, the interface between a pair of antibody molecules can be engineered to maximize the percentage of heterodimers which are recovered from recombinant-cell culture. The preferred interface comprises at least a part of the C<sub>H</sub>3 region of an antibody constant domain. In this method, one or more small amino acid side chains from the interface of the first antibody molecule are replaced with larger side chains (*e.g.*, tyrosine or tryptophan). Compensatory “cavities” of identical or similar size to the large side chains(s) are created on the interface of the second antibody molecule by replacing large amino acid side chains with smaller ones (*e.g.*, alanine or threonine). This provides a mechanism for increasing the yield of the heterodimer over other unwanted end-products such as homodimers.

**[0159]** Techniques for generating bispecific antibodies from antibody fragments have been described in the literature. For example, bispecific antibodies can be prepared using chemical linkage. Brennan et al., *Science* 229:81 (1985) describe a procedure wherein intact antibodies are

proteolytically cleaved to generate  $F(ab')_2$  fragments. These fragments are reduced in the presence of the dithiol complexing agent sodium arsenite to stabilize vicinal dithiols and prevent intermolecular disulfide formation. The Fab' fragments generated are then converted to thionitrobenzoate (TNB) derivatives. One of the Fab'-TNB derivatives is then reconverted to the Fab'-TNB derivative to form the bispecific antibody. The bispecific antibodies produced can be used as agents for the selective immobilization of enzymes.

[0160] Fab' fragments may be directly recovered from *E. coli* and chemically coupled to form bispecific antibodies. Shalaby et al., *J. Exp. Med.* 175: 217-225 (1992) describes the production of fully humanized bispecific antibody  $F(ab')_2$  molecules. Each Fab' fragment was separately secreted from *E. coli* and subjected to directed chemical coupling *in vitro* to form the bispecific antibody. The bispecific antibody thus formed was able to bind to cells overexpressing the ErbB2 receptor and normal human T-cells, as well as trigger the lytic activity of human cytotoxic lymphocytes against human breast tumor targets.

[0161] Various techniques for making and isolating bivalent antibody fragments directly from recombinant-cell culture have also been described. For example, bivalent heterodimers have been produced using leucine zippers. Kostelny et al., *J. Immunol.*, 148(5):1547-1553 (1992). The leucine zipper peptides from the Fos and Jun proteins were linked to the Fab' portions of two different antibodies by gene fusion. The antibody homodimers were reduced at the hinge region to form monomers and then re-oxidized to form the antibody heterodimers. The "diabody" technology described by Hollinger et al., *Proc. Nat'l Acad. Sci. USA*, 90: 6444-6448 (1993) has provided an alternative mechanism for making bispecific/bivalent antibody fragments. The fragments comprise a heavy-chain variable domain ( $V_H$ ) connected to a light-chain variable domain ( $V_L$ ) by a linker which is too short to allow pairing between the two domains on the same chain. Accordingly, the  $V_H$  and  $V_L$  domains of one fragment are forced to pair with the complementary  $V_L$  and  $V_H$  domains of another fragment, thereby forming two antigen-binding sites. Another strategy for making bispecific/bivalent antibody fragments by the use of single-chain Fv (sFv) dimers has also been reported. See Gruber et al., *J. Immunol.*, 152:5368 (1994).

[0162] Antibodies with more than two valencies are also contemplated. For example, trispecific antibodies can be prepared. Tutt et al., *J. Immunol.* 147:60 (1991).

[0163] Exemplary bispecific antibodies may bind to two different antigens. In some embodiments a bispecific antibody binds to a first antigen C1q, C1r, or C1s and a second antigen facilitating transport across the blood-brain barrier. Numerous antigens are known in the art that

facilitate transport across the blood-brain barrier (*see, e.g.*, Gabathuler R., Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases, *Neurobiol. Dis.* 37 (2010) 48-57). Such second antigens include, without limitation, transferrin receptor (TR), insulin receptor (HIR), Insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, including CRM197 (a non-toxic mutant of diphtheria toxin), llama single domain antibodies such as TMEM 30(A) (Flippase), protein transduction domains such as TAT, Syn-B, or penetratin, poly-arginine or generally positively charged peptides, and Angiopep peptides such as ANG1005 (*see, e.g.*, Gabathuler, 2010).

#### (7) Multivalent antibodies

**[0164]** A multivalent antibody may be internalized (and/or catabolized) faster than a bivalent antibody by a cell expressing an antigen to which the antibodies bind. The anti-C1s antibodies of the present disclosure or antibody fragments thereof can be multivalent antibodies (which are other than of the IgM class) with three or more antigen binding sites (*e.g.*, tetravalent antibodies), which can be readily produced by recombinant expression of nucleic acid encoding the polypeptide chains of the antibody. The multivalent antibody can comprise a dimerization domain and three or more antigen binding sites. The preferred dimerization domain comprises an Fc region or a hinge region. In this scenario, the antibody will comprise an Fc region and three or more antigen binding sites amino-terminal to the Fc region. The preferred multivalent antibody herein contains three to about eight, but preferably four, antigen binding sites. The multivalent antibody contains at least one polypeptide chain (and preferably two polypeptide chains), wherein the polypeptide chain or chains comprise two or more variable domains. For instance, the polypeptide chain or chains may comprise VD1-(X1)<sub>n</sub>-VD2-(X2)<sub>n</sub>-Fc, wherein VD1 is a first variable domain, VD2 is a second variable domain, Fc is one polypeptide chain of an Fc region, X1 and X2 represent an amino acid or polypeptide, and n is 0 or 1. Similarly, the polypeptide chain or chains may comprise V<sub>H</sub>-C<sub>H</sub>1-flexible linker-V<sub>H</sub>-C<sub>H</sub>1-Fc region chain; or V<sub>H</sub>-C<sub>H</sub>1-V<sub>H</sub>-C<sub>H</sub>1-Fc region chain. The multivalent antibody herein preferably further comprises at least two (and preferably four) light chain variable domain polypeptides. The multivalent antibody herein may, for instance, comprise from about two to about eight light chain variable domain polypeptides. The light chain variable domain polypeptides contemplated here comprise a light chain variable domain and, optionally, further comprise a CL domain.

(8) *Heteroconjugate antibodies*

[0165] Heteroconjugate antibodies are also within the scope of the present disclosure.

Heteroconjugate antibodies are composed of two covalently joined antibodies (*e.g.*, anti-C1s antibodies of the present disclosure or antibody fragments thereof). For example, one of the antibodies in the heteroconjugate can be coupled to avidin, the other to biotin. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells, U.S. Patent No. 4,676,980, and have been used to treat HIV infection. International Publication Nos. WO 91/00360, WO 92/200373 and EP 0308936. It is contemplated that the antibodies may be prepared *in vitro* using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins may be constructed using a disulfide exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptobutyrimidate and those disclosed, for example, in U.S. Patent No. 4,676,980. Heteroconjugate antibodies may be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

(9) *Effector function engineering*

[0166] It may also be desirable to modify an anti-C1s antibody of the present disclosure to modify effector function and/or to increase serum half-life of the antibody. For example, the Fc receptor binding site on the constant region may be modified or mutated to remove or reduce binding affinity to certain Fc receptors, such as Fc $\gamma$ RI, Fc $\gamma$ RII, and/or Fc $\gamma$ RIII. In some embodiments, the effector function is impaired by removing N-glycosylation of the Fc region (*e.g.*, in the CH 2 domain of IgG) of the antibody. In some embodiments, the effector function is impaired by modifying regions such as 233-236, 297, and/or 327-331 of human IgG as described in PCT WO 99/58572 and Armour et al., *Molecular Immunology* 40: 585-593 (2003); Reddy et al., *J. Immunology* 164:1925-1933 (2000).

[0167] The constant region of the anti-complement antibodies described herein may also be modified to impair complement activation. For example, complement activation of IgG antibodies following binding of the C1 component of complement may be reduced by mutating amino acid residues in the constant region in a C1 binding motif (*e.g.*, C1s binding motif). It has been reported that Ala mutation for each of D270, K322, P329, P331 of human IgG1 significantly reduced the ability of the antibody to bind to C1s and activating complement. For murine IgG2b, C1s binding motif constitutes residues E318, K320, and K322. Idusogie *et al.*

(2000) *J. Immunology* 164:4178-4184; Duncan *et al.* (1988) *Nature* 322: 738-740. As the C1s binding motif E318, K320, and K322 identified for murine IgG2b is believed to be common for other antibody isotypes (Duncan *et al.* (1988) *Nature* 322:738-740), C1s binding activity for IgG2b can be abolished by replacing any one of the three specified residues with a residue having an inappropriate functionality on its side chain. It is not necessary to replace the ionic residues only with Ala to abolish C1s binding. It is also possible to use other alkyl-substituted non-ionic residues, such as Gly, Ile, Leu, or Val, or such aromatic non-polar residues as Phe, Tyr, Trp and Pro in place of any one of the three residues in order to abolish C1q binding. In addition, it is also possible to use such polar non-ionic residues as Ser, Thr, Cys, and Met in place of residues 320 and 322, but not 318, in order to abolish C1s binding activity. In addition, removal of carbohydrate modifications of the Fc region necessary for complement binding can prevent complement activation. Glycosylation of a conserved asparagine (Asn-297) on the CH2 domain of IgG heavy chains is essential for antibody effector functions (Jefferis *et al.* (1998) *Immunol Rev* 163:59–76). Modification of the Fc glycan alters IgG conformation and reduces the Fc affinity for binding of complement protein C1s and effector cell receptor FcR (Alhorn *et al.* (2008) *PLoS ONE* 2008;3:e1413). Complete removal of the Fc glycan abolishes CDC and ADCC. Deglycosylation can be performed using glycosidase enzymes for example Endoglycosidase S (EndoS), a 108kDa enzyme encoded by the gene *endoS* of *Streptococcus pyogenes* that selectively digests asparagine-linked glycans on the heavy chain of all IgG subclasses, without action on other immunoglobulin classes or other glycoproteins (Collin *et al.* (2001) *EMBO J* 2001;20:3046–3055).

**[0168]** To increase the serum half-life of the antibody, one may incorporate a salvage receptor binding epitope into the antibody (especially an antibody fragment) as described in U.S. Patent 5,739,277, for example. As used herein, the term “*salvage receptor binding epitope*” refers to an epitope of the Fc region of an IgG molecule (*e.g.*, IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub>) that is responsible for increasing the *in vivo* serum half-life of the IgG molecule.

(10) *Other amino acid sequence modifications*

**[0169]** Amino acid sequence modifications of anti-C1s antibodies of the present disclosure, or antibody fragments thereof, are also contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibodies or antibody fragments. Amino acid sequence variants of the antibodies or antibody fragments are prepared by introducing appropriate nucleotide changes into the nucleic acid encoding the antibodies or

antibody fragments, or by peptide synthesis. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics (*i.e.*, the ability to bind or physically interact with a C1s protein of the present disclosure). The amino acid changes also may alter post-translational processes of the antibody, such as changing the number or position of glycosylation sites.

**[0170]** A useful method for identification of certain residues or regions of the anti-C1s antibody that are preferred locations for mutagenesis is called “alanine scanning mutagenesis” as described by Cunningham and Wells in *Science*, 244:1081-1085 (1989). Here, a residue or group of target residues are identified (*e.g.*, charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the target antigen. Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* need not be predetermined. For example, to analyze the performance of a mutation at a given site, alanine scanning or random mutagenesis is conducted at the target codon or region and the expressed antibody variants are screened for the desired activity.

**[0171]** Amino acid sequence insertions include amino- (“N”) and/or carboxy- (“C”) terminal fusions ranging in length from one residue to polypeptides containing a hundred or more residues, as well as intrasequence insertions of single or multiple amino acid residues. Examples of terminal insertions include an antibody with an N-terminal methionyl residue or the antibody fused to a cytotoxic polypeptide. Other insertional variants of the antibody molecule include the fusion to the N- or C-terminus of the antibody to an enzyme or a polypeptide which increases the serum half-life of the antibody.

**[0172]** Another type of variant is an amino acid substitution variant. These variants have at least one amino acid residue in the antibody molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated. Conservative substitutions are shown in the Table A below under the heading of “preferred substitutions”. If such substitutions result in a change in biological activity, then more substantial changes, denominated “exemplary substitutions” in

Table A, or as further described below in reference to amino acid classes, may be introduced and the products screened.

**TABLE A: Amino Acid Substitutions**

| Original Residue | Exemplary Substitutions             | Preferred Substitutions |
|------------------|-------------------------------------|-------------------------|
| Ala (A)          | val; leu; ile                       | val                     |
| Arg (R)          | lys; gln; asn                       | lys                     |
| Asn (N)          | gln; his; asp, lys; arg             | gln                     |
| Asp (D)          | glu; asn                            | glu                     |
| Cys (C)          | ser; ala                            | ser                     |
| Gln (Q)          | asn; glu                            | asn                     |
| Glu (E)          | asp; gln                            | asp                     |
| Gly (G)          | ala                                 | ala                     |
| His (H)          | asn; gln; lys; arg                  | arg                     |
| Ile (I)          | leu; val; met; ala; phe; norleucine | leu                     |
| Leu (L)          | norleucine; ile; val; met; ala; phe | ile                     |
| Lys (K)          | arg; gln; asn                       | arg                     |
| Met (M)          | leu; phe; ile                       | leu                     |
| Phe (F)          | leu; val; ile; ala; tyr             | tyr                     |
| Pro (P)          | ala                                 | ala                     |
| Ser (S)          | thr                                 | thr                     |
| Thr (T)          | ser                                 | ser                     |
| Trp (W)          | tyr; phe                            | tyr                     |
| Tyr (Y)          | trp; phe; thr; ser                  | phe                     |
| Val (V)          | ile; leu; met; phe; ala; norleucine | leu                     |

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**[0173]** Substantial modifications in the biological properties of the antibody are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c)

the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties:

- (1) hydrophobic: norleucine, met, ala, val, leu, ile;
- (2) neutral hydrophilic: cys, ser, thr;
- 5 (3) acidic: asp, glu;
- (4) basic: asn, gln, his, lys, arg;
- (5) residues that influence chain orientation: gly, pro; and
- (6) aromatic: trp, tyr, phe.

**[0174]** Non-conservative substitutions entail exchanging a member of one of these classes  
10 for another class.

**[0175]** Any cysteine residue not involved in maintaining the proper conformation of the antibody also may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment, such as  
15 an Fv fragment).

**[0176]** A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (*e.g.* a humanized or human anti-C1s antibody). Generally, the resulting variant(s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A  
20 convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their  
25 biological activity (*e.g.*, binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding.

Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen (*e.g.*, a C1s  
30 protein of the present disclosure). Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated,

the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0177] Another type of amino acid variant of the antibody alters the original glycosylation pattern of the antibody. By altering is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

[0178] Glycosylation of antibodies is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acylgalactosamine, galactose, or xylose to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[0179] Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or substitution by, one or more serine or threonine residues to the sequence of the original antibody (for O-linked glycosylation sites).

[0180] Nucleic acid molecules encoding amino acid sequence variants of the anti-IgE antibody are prepared by a variety of methods known in the art. These methods include, but are not limited to, isolation from a natural source (in the case of naturally occurring amino acid sequence variants) or preparation by oligonucleotide-mediated (or site-directed) mutagenesis, PCR mutagenesis, and cassette mutagenesis of an earlier prepared variant or a non-variant version of the antibodies (*e.g.*, anti-C1s antibody of the present disclosure) or antibody fragments.

(11) *Other antibody modifications*

[0181] Anti-C1s antibodies of the present disclosure, or antibody fragments thereof, can be further modified to contain additional non-proteinaceous moieties that are known in the art and readily available. Preferably, the moieties suitable for derivatization of the antibody are water-soluble polymers. Non-limiting examples of water-soluble polymers include, but are not limited

to, polyethylene glycol (PEG), copolymers of ethylene glycol/propylene glycol, carboxymethylcellulose, dextran, polyvinyl alcohol, polyvinyl pyrrolidone, poly-1, 3-dioxolane, poly-1,3,6-trioxane, ethylene/maleic anhydride copolymer, polyaminoacids (either homopolymers or random copolymers), and dextran or poly(n-vinyl pyrrolidone)polyethylene glycol, polypropylene glycol homopolymers, polypropylene oxide/ethylene oxide co-polymers, polyoxyethylated polyols (*e.g.*, glycerol), polyvinyl alcohol, and mixtures thereof. Polyethylene glycol propionaldehyde may have advantages in manufacturing due to its stability in water. The polymer may be of any molecular weight, and may be branched or unbranched. The number of polymers attached to the antibody may vary, and if more than one polymer is attached, they can be the same or different molecules. In general, the number and/or type of polymers used for derivatization can be determined based on considerations including, but not limited to, the particular properties or functions of the antibody to be improved, whether the antibody derivative will be used in a therapy under defined conditions, *etc.* Such techniques and other suitable formulations are disclosed in *Remington: The Science and Practice of Pharmacy*, 20th Ed., Alfonso Gennaro, Ed., Philadelphia College of Pharmacy and Science (2000).

#### **Nucleic acids, vectors, and host cells**

**[0182]** Anti-C1s antibodies of the present disclosure may be produced using recombinant methods and compositions, *e.g.*, as described in U.S. Patent No. 4,816,567. In some embodiments, isolated nucleic acids having a nucleotide sequence encoding any of the anti-C1s antibodies of the present disclosure are provided. Such nucleic acids may encode an amino acid sequence containing the VL and/or an amino acid sequence containing the VH of the anti-C1s antibody (*e.g.*, the light and/or heavy chains of the antibody). In some embodiments, one or more vectors (*e.g.*, expression vectors) containing such nucleic acids are provided. In some embodiments, a host cell containing such nucleic acid is also provided. In some embodiments, the host cell contains (*e.g.*, has been transduced with): (1) a vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and an amino acid sequence containing the VH of the antibody, or (2) a first vector containing a nucleic acid that encodes an amino acid sequence containing the VL of the antibody and a second vector containing a nucleic acid that encodes an amino acid sequence containing the VH of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell).

[0183] Methods of making an anti-C1s antibody of the present disclosure are provided. In some embodiments, the method includes culturing a host cell of the present disclosure containing a nucleic acid encoding the anti-C1s antibody, under conditions suitable for expression of the antibody. In some embodiments, the antibody is subsequently recovered from the host cell (or host cell culture medium).

[0184] For recombinant production of an anti-C1s antibody of the present disclosure, a nucleic acid encoding the anti-C1s antibody is isolated and inserted into one or more vectors for further cloning and/or expression in a host cell. Such nucleic acid may be readily isolated and sequenced using conventional procedures (*e.g.*, by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody).

[0185] Suitable vectors containing a nucleic acid sequence encoding any of the anti-C1s antibodies of the present disclosure, or fragments thereof polypeptides (including antibodies) described herein include, without limitation, cloning vectors and expression vectors. Suitable cloning vectors can be constructed according to standard techniques, or may be selected from a large number of cloning vectors available in the art. While the cloning vector selected may vary according to the host cell intended to be used, useful cloning vectors generally have the ability to self-replicate, may possess a single target for a particular restriction endonuclease, and/or may carry genes for a marker that can be used in selecting clones containing the vector. Suitable examples include plasmids and bacterial viruses, *e.g.*, pUC18, pUC19, Bluescript (*e.g.*, pBS SK+) and its derivatives, mpl8, mpl9, pBR322, pMB9, ColE1, pCR1, RP4, phage DNAs, and shuttle vectors such as pSA3 and pAT28. These and many other cloning vectors are available from commercial vendors such as BioRad, Strategene, and Invitrogen.

[0186] Expression vectors generally are replicable polynucleotide constructs that contain a nucleic acid of the present disclosure. The expression vector may be replicable in the host cells either as episomes or as an integral part of the chromosomal DNA. Suitable expression vectors include but are not limited to plasmids, viral vectors, including adenoviruses, adeno-associated viruses, retroviruses, cosmids, and expression vector(s) disclosed in PCT Publication No. WO 87/04462. Vector components may generally include, but are not limited to, one or more of the following: a signal sequence; an origin of replication; one or more marker genes; suitable transcriptional controlling elements (such as promoters, enhancers and terminator). For expression (*i.e.*, translation), one or more translational controlling elements are also usually required, such as ribosome binding sites, translation initiation sites, and stop codons.

[0187] The vectors containing the nucleic acids of interest can be introduced into the host cell by any of a number of appropriate means, including electroporation, transfection employing calcium chloride, rubidium chloride, calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; and infection (*e.g.*, where the vector is an infectious agent such as vaccinia virus). The choice of introducing vectors or polynucleotides will often depend on features of the host cell. In some embodiments, the vector contains a nucleic acid containing one or more amino acid sequences encoding an anti-C1s antibody of the present disclosure.

[0188] Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, anti-C1s antibodies of the present disclosure may be produced in bacteria, in particular when glycosylation and Fc effector function are not needed. For expression of antibody fragments and polypeptides in bacteria (*e.g.*, U.S. Patent Nos. 5,648,237, 5,789,199, and 5,840,523; and Charlton, *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ, 2003), pp. 245-254, describing expression of antibody fragments in *E. coli*). After expression, the antibody may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

[0189] In addition to prokaryotes, eukaryotic microorganisms, such as filamentous fungi or yeast, are also suitable cloning or expression hosts for antibody-encoding vectors, including fungi and yeast strains whose glycosylation pathways have been “humanized,” resulting in the production of an antibody with a partially or fully human glycosylation pattern (*e.g.*, Gerngross, *Nat. Biotech.* 22:1409-1414 (2004); and Li et al., *Nat. Biotech.* 24:210-215 (2006)).

[0190] Suitable host cells for the expression of glycosylated antibody can also be derived from multicellular organisms (invertebrates and vertebrates). Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains have been identified which may be used in conjunction with insect cells, particularly for transfection of *Spodoptera frugiperda* cells. Plant cell cultures can also be utilized as hosts (*e.g.*, U.S. Patent Nos. 5,959,177, 6,040,498, 6,420,548, 7,125,978, and 6,417,429, describing PLANTIBODIES™ technology for producing antibodies in transgenic plants.).

[0191] Vertebrate cells may also be used as hosts. For example, mammalian cell lines that are adapted to grow in suspension may be useful. Other examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7); human embryonic kidney line (293 or 293 cells as described, *e.g.*, in Graham et al., *J. Gen Virol.* 36:59 (1977)); baby hamster

kidney cells (BHK); mouse sertoli cells (TM4 cells as described, *e.g.*, in Mather, *Biol. Reprod.* 23:243-251 (1980)); monkey kidney cells (CV1); African green monkey kidney cells (VERO-76); human cervical carcinoma cells (HELA); canine kidney cells (MDCK; buffalo rat liver cells (BRL 3A); human lung cells (W138); human liver cells (Hep G2); mouse mammary tumor  
5 (MMT 060562); TRI cells, as described, *e.g.*, in Mather et al., *Annals N.Y. Acad. Sci.* 383:44-68 (1982); MRC 5 cells; and FS4 cells. Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR- CHO cells (Urlaub et al., *Proc. Natl. Acad. Sci. USA* 77:4216 (1980)); and myeloma cell lines such as Y0, NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, *e.g.*, Yazaki and Wu,  
10 *Methods in Molecular Biology*, Vol. 248 (B.K.C. Lo, ed., Humana Press, Totowa, NJ), pp. 255-268 (2003).

### Pharmaceutical compositions

[0192] Anti-C1s antibodies of the present disclosure can be incorporated into a variety of formulations for therapeutic use (*e.g.*, by administration) or in the manufacture of a medicament  
15 (*e.g.*, for treating or preventing an autoimmune or neurodegenerative disease) by combining the antibodies with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms. Examples of such formulations include, without limitation, tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. Pharmaceutical  
20 compositions can include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are vehicles commonly used to formulate pharmaceutical compositions for animal or human administration. The diluent is selected so as not to affect the biological activity of the combination. Examples of such diluents include, without limitation, distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution,  
25 and Hank's solution. A pharmaceutical composition or formulation of the present disclosure can further include other carriers, adjuvants, or non-toxic, nontherapeutic, nonimmunogenic stabilizers, excipients and the like. The compositions can also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

30 [0193] A pharmaceutical composition of the present disclosure can also include any of a variety of stabilizing agents, such as an antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide can be complexed with various well-known

compounds that enhance the *in vivo* stability of the polypeptide, or otherwise enhance its pharmacological properties (*e.g.*, increase the half-life of the polypeptide, reduce its toxicity, and enhance solubility or uptake). Examples of such modifications or complexing agents include, without limitation, sulfate, gluconate, citrate and phosphate. The polypeptides of a composition can also be complexed with molecules that enhance their *in vivo* attributes. Such molecules include, without limitation, carbohydrates, polyamines, amino acids, other peptides, ions (*e.g.*, sodium, potassium, calcium, magnesium, manganese), and lipids.

[0194] Further examples of formulations that are suitable for various types of administration can be found in Remington's *Pharmaceutical Sciences*, Mace Publishing Company, Philadelphia, PA, 17th ed. (1985). For a brief review of methods for drug delivery, see, Langer, *Science* 249:1527-1533 (1990).

[0195] For oral administration, the active ingredient can be administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. The active component(s) can be encapsulated in gelatin capsules together with inactive ingredients and powdered carriers, such as glucose, lactose, sucrose, mannitol, starch, cellulose or cellulose derivatives, magnesium stearate, stearic acid, sodium saccharin, talcum, magnesium carbonate. Examples of additional inactive ingredients that may be added to provide desirable color, taste, stability, buffering capacity, dispersion or other known desirable features are red iron oxide, silica gel, sodium lauryl sulfate, titanium dioxide, and edible white ink.

Similar diluents can be used to make compressed tablets. Both tablets and capsules can be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets can be sugar coated or film coated to mask any unpleasant taste and protect the tablet from the atmosphere, or enteric-coated for selective disintegration in the gastrointestinal tract. Liquid dosage forms for oral administration can contain coloring and flavoring to increase patient acceptance.

[0196] Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient, and aqueous and non-aqueous sterile suspensions that can include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

[0197] The components used to formulate the pharmaceutical compositions are preferably of high purity and are substantially free of potentially harmful contaminants (*e.g.*, at least National

Food (NF) grade, generally at least analytical grade, and more typically at least pharmaceutical grade). Moreover, compositions intended for *in vivo* use are usually sterile. To the extent that a given compound must be synthesized prior to use, the resulting product is typically substantially free of any potentially toxic agents, particularly any endotoxins, which may be present during the synthesis or purification process. Compositions for parental administration are also sterile, substantially isotonic and made under GMP conditions.

**[0198]** Formulations may be optimized for retention and stabilization in the brain or central nervous system. When the agent is administered into the cranial compartment, it is desirable for the agent to be retained in the compartment, and not to diffuse or otherwise cross the blood brain barrier. Stabilization techniques include cross-linking, multimerizing, or linking to groups such as polyethylene glycol, polyacrylamide, neutral protein carriers, *etc.* in order to achieve an increase in molecular weight.

**[0199]** Other strategies for increasing retention include the entrapment of the antibody, such as an anti-C1s antibody of the present disclosure, in a biodegradable or bioerodible implant. The rate of release of the therapeutically active agent is controlled by the rate of transport through the polymeric matrix, and the biodegradation of the implant. The transport of drug through the polymer barrier will also be affected by compound solubility, polymer hydrophilicity, extent of polymer cross-linking, expansion of the polymer upon water absorption so as to make the polymer barrier more permeable to the drug, geometry of the implant, and the like. The implants are of dimensions commensurate with the size and shape of the region selected as the site of implantation. Implants may be particles, sheets, patches, plaques, fibers, microcapsules and the like and may be of any size or shape compatible with the selected site of insertion.

**[0200]** The implants may be monolithic, *i.e.* having the active agent homogeneously distributed through the polymeric matrix, or encapsulated, where a reservoir of active agent is encapsulated by the polymeric matrix. The selection of the polymeric composition to be employed will vary with the site of administration, the desired period of treatment, patient tolerance, the nature of the disease to be treated and the like. Characteristics of the polymers will include biodegradability at the site of implantation, compatibility with the agent of interest, ease of encapsulation, a half-life in the physiological environment.

**[0201]** Biodegradable polymeric compositions which may be employed may be organic esters or ethers, which when degraded result in physiologically acceptable degradation products, including the monomers. Anhydrides, amides, orthoesters or the like, by themselves or in

combination with other monomers, may find use. The polymers will be condensation polymers. The polymers may be cross-linked or non-cross-linked. Of particular interest are polymers of hydroxyaliphatic carboxylic acids, either homo- or copolymers, and polysaccharides. Included among the polyesters of interest are polymers of D-lactic acid, L-lactic acid, racemic lactic acid, glycolic acid, polycaprolactone, and combinations thereof. By employing the L-lactate or D-lactate, a slowly biodegrading polymer is achieved, while degradation is substantially enhanced with the racemate. Copolymers of glycolic and lactic acid are of particular interest, where the rate of biodegradation is controlled by the ratio of glycolic to lactic acid. The most rapidly degraded copolymer has roughly equal amounts of glycolic and lactic acid, where either homopolymer is more resistant to degradation. The ratio of glycolic acid to lactic acid will also affect the brittleness of in the implant, where a more flexible implant is desirable for larger geometries. Among the polysaccharides of interest are calcium alginate, and functionalized celluloses, particularly carboxymethylcellulose esters characterized by being water insoluble, a molecular weight of about 5 kD to 500 kD, *etc.* Biodegradable hydrogels may also be employed in the implants of the subject invention. Hydrogels are typically a copolymer material, characterized by the ability to imbibe a liquid. Exemplary biodegradable hydrogels which may be employed are described in Heller in: *Hydrogels in Medicine and Pharmacy*, N. A. Peppes ed., Vol. III, CRC Press, Boca Raton, Fla., 1987, pp 137-149.

#### *Pharmaceutical dosages*

**[0202]** Pharmaceutical compositions of the present disclosure containing an anti-C1s antibody of the present disclosure may be used (*e.g.*, administered to an individual in need of treatment with anti-C1s antibody, preferably a human), in accordance with known methods, such as intravenous administration as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intracerebrospinal, intracranial, intraspinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes.

**[0203]** Dosages and desired drug concentration of pharmaceutical compositions of the present disclosure may vary depending on the particular use envisioned. The determination of the appropriate dosage or route of administration is well within the skill of an ordinary artisan. Animal experiments provide reliable guidance for the determination of effective doses for human therapy. Interspecies scaling of effective doses can be performed following the principles described in Mordenti, J. and Chappell, W. "The Use of Interspecies Scaling in Toxicokinetics,"

In *Toxicokinetics and New Drug Development*, Yacobi et al., Eds, Pergamon Press, New York 1989, pp.42-46.

[0204] For *in vivo* administration of any of the anti-C1s antibodies of the present disclosure, normal dosage amounts may vary from about 10 ng/kg up to about 100 mg/kg of an individual's body weight or more per day, preferably about 1 mg/kg/day to 10 mg/kg/day, depending upon the route of administration. For repeated administrations over several days or longer, depending on the severity of the disease, disorder, or condition to be treated, the treatment is sustained until a desired suppression of symptoms is achieved.

[0205] An exemplary dosing regimen may include administering an initial dose of an anti-C1s antibody, of about 2 mg/kg, followed by a weekly maintenance dose of about 1 mg/kg every other week. Other dosage regimens may be useful, depending on the pattern of pharmacokinetic decay that the physician wishes to achieve. For example, dosing an individual from one to twenty-one times a week is contemplated herein. In certain embodiments, dosing ranging from about 3 µg/kg to about 2 mg/kg (such as about 3 µg/kg, about 10 µg/kg, about 30 µg/kg, about 100 µg/kg, about 300 µg/kg, about 1 mg/kg, or about 2 mg/kg) may be used. In certain embodiments, dosing frequency is three times per day, twice per day, once per day, once every other day, once weekly, once every two weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, once every eight weeks, once every nine weeks, once every ten weeks, or once monthly, once every two months, once every three months, or longer. Progress of the therapy is easily monitored by conventional techniques and assays. The dosing regimen, including the anti-C1s antibody administered, can vary over time independently of the dose used.

[0206] Dosages for a particular anti-C1s antibody may be determined empirically in individuals who have been given one or more administrations of the anti-C1s antibody.

Individuals are given incremental doses of an anti-C1s antibody. To assess efficacy of an anti-C1s antibody, any clinical symptom of NMO can be monitored.

[0207] Administration of an anti-C1s antibody of the present disclosure can be continuous or intermittent, depending, for example, on the recipient's physiological condition, whether the purpose of the administration is therapeutic or prophylactic, and other factors known to skilled practitioners. The administration of an anti-C1s antibody may be essentially continuous over a preselected period of time or may be in a series of spaced doses.

[0208] Guidance regarding particular dosages and methods of delivery is provided in the literature; see, for example, U.S. Patent Nos. 4,657,760; 5,206,344; or 5,225,212. It is within the scope of the invention that different formulations will be effective for different treatments and different disorders, and that administration intended to treat a specific organ or tissue may necessitate delivery in a manner different from that to another organ or tissue. Moreover, dosages may be administered by one or more separate administrations, or by continuous infusion. For repeated administrations over several days or longer, depending on the condition, the treatment is sustained until a desired suppression of disease symptoms occurs. However, other dosage regimens may be useful. The progress of this therapy is easily monitored by conventional techniques and assays.

### Therapeutic uses

[0209] The present disclosure provides anti-C1s antibodies, and antigen-binding fragments thereof, which can bind to and neutralize a biologic activity of C1s. These anti-C1s antibodies are useful for preventing, reducing risk, or treating a wide range of autoimmune and neurodegenerative diseases, including without limitation, Neuromyelitis Optica, Bullous Pemphigoid, Alzheimer's Disease, Glaucoma, Spinal Muscular Atrophy, Stroke, Traumatic Brain Injury, Multiple Sclerosis (MS), Age-related Macular Degeneration (AMD), and even provide benefits in the normal aging process.

[0210] In autoimmune diseases, such as NMO, autoantibodies activate the complement system. In neurodegenerative diseases, such as Alzheimer's Disease and Glaucoma, complement factors, such as complement factor C1 and its subunits such as C1q, were shown to be expressed in neurons, where they acting as signals for synapse elimination. *See, e.g.*, U.S. Patent Publication Nos. US 2012/0195880 and US 2012/0328601. Accordingly, the neutralizing anti-C1s antibodies of this disclosure are useful for preventing, reducing or treating synaptic loss in neurodegenerative disorders, including pathological C1-dependent synaptic loss. The anti-C1s antibodies are further useful to prevent or reduce pathological activity-dependent synaptic pruning, synapse phagocytosis by microglia and synaptic loss that is dependent on the complement receptor 3 (CR3)/C3.

[0211] In NMO patients, the classical complement pathway is triggered by the binding of an autoantibody, such as an AQP4-targeted autoantibody, to its autoantigen. An anti-C1s antibody of this disclosure may be administered to cells *in vitro* to prevent complement-dependent cytotoxicity or complement-dependent cell-mediated cytotoxicity. An anti-C1s antibody may

also be administered in *ex vivo* spinal cord slice models of NMO. Alternatively, the anti-C1s antibody may be administered *in vivo* (e.g., by administering the antibody to an individual, such as a murine or human individual) to prevent lesion formation (as indicated, e.g., by loss of AQP4, GFAP, or myelin).

## 5 *Combination Treatments*

**[0212]** The antibodies of the present disclosure may be used, without limitation, in combination with any additional treatment for autoimmune and/or neurodegenerative diseases, such as NMO, including, without limitation, immunosuppressive therapies.

**[0213]** In some embodiments, an anti-C1s antibody of this disclosure is administered in  
10 therapeutically effective amounts in combination with a second neutralizing anti-complement factor antibody, such as an anti-C1q or anti-C1r antibody, or a second anti-C1s antibody. In some embodiments, an anti-C1s antibody of this disclosure is administered in therapeutically effective amounts with a second and a third neutralizing anti-complement factor antibody, such as a second anti-C1s antibody, an anti-C1q, and/or an anti-C1r antibody.

**[0214]** In some embodiments, the anti-C1s antibodies of this disclosure are administered in  
15 combination with an inhibitor of antibody-dependent cellular cytotoxicity (ADCC). ADCC inhibitors may include, without limitation, soluble NK cell inhibitory receptors such as the killer cell Ig-like receptors (KIRs), which recognize HLA-A, HLA-B, or HLA-C and C-type lectin CD94/NKG2A heterodimers, which recognize HLA-E (*see, e.g.,* López-Botet M., T. Bellón, M. Llano, F. Navarro, P. García & M. de Miguel. (2000), Paired inhibitory and triggering NK cell  
20 receptors for HLA class I molecules. *Hum. Immunol.* **61**: 7-17; Lanier L.L. (1998) Follow the leader: NK cell receptors for classical and nonclassical MHC class I. *Cell* **92**: 705-707.), and cadmium (*see, e.g.,* Immunopharmacology 1990; Volume 20, Pages 73-8).

**[0215]** In some embodiments, the antibodies of this disclosure are administered in  
25 combination with an inhibitor of the alternative pathway of complement activation. Such inhibitors may include, without limitation, factor B blocking antibodies, factor D blocking antibodies, soluble, membrane-bound, tagged or fusion-protein forms of CD59, DAF, CR1, CR2, Crry or Comstatin-like peptides that block the cleavage of C3, non-peptide C3aR antagonists such as SB 290157, Cobra venom factor or non-specific complement inhibitors such  
30 as nafamostat mesilate (FUTHAN; FUT-175), aprotinin, K-76 monocarboxylic acid (MX-1) and heparin (*see, e.g.,* T.E. Mollnes & M. Kirschfink, *Molecular Immunology* 43 (2006) 107-121). In some embodiments, the antibodies of this disclosure are administered in combination with an

inhibitor of the interaction between the autoantibody and its autoantigen. Such inhibitors may include purified soluble forms of the autoantigen, or antigen mimetics such as peptide or RNA-derived mimotopes, including mimotopes of the AQP4 antigen. Alternatively, such inhibitors may include blocking agents that recognize the autoantigen and prevent binding of the autoantibody without triggering the classical complement pathway. Such blocking agents may include, *e.g.*, autoantigen-binding RNA aptamers or antibodies lacking functional C1q binding sites in their Fc domains (*e.g.*, Fab fragments or antibody otherwise engineered not to bind C1q).

### **Kits**

[0216] The invention also provides kits containing antibodies of this disclosure, and functional fragments thereof. Kits of the invention include one or more containers comprising a purified anti-C1s antibody and instructions for use in accordance with methods known in the art. Generally, these instructions comprise a description of administration of the inhibitor to treat or diagnose, *e.g.*, NMO (such as an antibody of this disclosure), according to any methods known in the art. The kit may further comprise a description of selecting an individual suitable for treatment based on identifying whether that individual has the disease and the stage of the disease.

[0217] The instructions generally include information as to dosage, dosing schedule, and route of administration for the intended treatment. The containers may be unit doses, bulk packages (*e.g.*, multi-dose packages) or sub-unit doses. Instructions supplied in the kits of the invention are typically written instructions on a label or package insert (*e.g.*, a paper sheet included in the kit), but machine-readable instructions (*e.g.*, instructions carried on a magnetic or optical storage disk) are also acceptable.

[0218] The label or package insert indicates that the composition is used for treating NMO. Instructions may be provided for practicing any of the methods described herein.

[0219] The kits of this invention are in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed Mylar or plastic bags), and the like. Also contemplated are packages for use in combination with a specific device, such as an inhaler, nasal administration device (*e.g.*, an atomizer) or an infusion device such as a minipump. A kit may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). The container may also have a sterile access port (*e.g.*, the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). At least one

active agent in the composition is an inhibitor of classical complement pathway. The container may further comprise a second pharmaceutically active agent.

[0220] Kits may optionally provide additional components such as buffers and interpretive information. Normally, the kit comprises a container and a label or package insert(s) on or  
5 associated with the container.

### Diagnostic Uses

[0221] The antibodies of this disclosure e, or functional fragments thereof, also have diagnostic utility. This disclosure therefore provides for methods of using the antibodies of this disclosure, or functional fragments thereof, for diagnostic purposes, including the detection of  
10 C1s in tissues, including tissues of a human patient. The anti-C1s antibodies of this disclosure are further useful for detecting synapses and synapse loss, *e.g.*, synapse loss experienced by patients suffering from autoimmune diseases or neurodegenerative disorders such as Alzheimer's Disease or Glaucoma. The phenomenon of synapse loss in neurodegeneration is well understood in the art. *See, e.g.*, U.S. Patent Publication Nos. 2012/0195880 and 2012/0328601.

[0222] In some embodiments, the diagnostic methods involve the administration of an anti-C1s antibody of this disclosure, or functional fragment thereof, to an individual and the detection of antibody levels bound to the synapses of the individual. Antibody binding to the synapses of an individual can be quantitatively measured by non-invasive techniques such as positron  
15 emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT).

[0223] In some embodiments, the diagnostic methods involve detecting synapses in a biological sample, such as a biopsy specimen, a tissue, or a cell. An anti-C1s antibody, or functional fragment thereof, is contacted with the biological sample and the level of antibody bound to the synapses present in the biological sample is then detected. The detection can be  
25 quantitative. Antibody detection in biological samples may occur with any method known in the art, including immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis or immunoprecipitation.

[0224] Quantitation of synapse-bound antibodies provides a relative quantitative measure for the number of synapses present in the individual. The diagnostic methods are typically repeated  
30 on a regular basis, whereas the exact periodicity of the diagnostic measurement depends on many factors, including the nature of the neurodegenerative disease, the stage of disease progression, treatment modalities and many other factors. Repeat diagnostic measurements typically reveal

progressive synapse loss in patients suffering from neurodegenerative diseases. Synapse loss may be followed over time in individual patients suffering from neurodegenerative diseases, but it may also be determined in a diseased patient population relative to a healthy patient population at any single point in time. Where patients are undergoing a specific therapy, the relative loss of synapse numbers in individuals undergoing the specific therapy relative to the synapse loss observed in patients not undergoing any treatments or undergoing control treatments can be used to assess the efficacy of the specific therapy provided.

[0225] The invention will be more fully understood by reference to the following Examples. They should not, however, be construed as limiting the scope of the invention. All citations throughout the disclosure are hereby expressly incorporated by reference.

## EXAMPLES

### Example 1: Production of Anti-C1s Antibodies

[0226] The anti-C1s antibodies of this disclosure, including 5A1 and 5C12 (also referred to as C1s<sup>mAb1</sup> and C1s<sup>mAb2</sup>), were generated by immunizing mice with human C1s enzyme purified from human plasma (Complement Technology Inc. Tyler Texas, Cat # A103) using standard mouse immunization and hybridoma screening technologies (Milstein, C (1999). *Bioessays* 21: 966–73; Mark Page, Robin Thorpe, The Protein Protocols Handbook 2002, Editors: John M. Walker, pp 1111-1113). In brief, female BALB/c mice were injected intraperitoneal with 25 µg of protein in complete Freund's adjuvant (CFA) on Day 0 and boosts were done with 25 µg of C1s enzyme in incomplete Freund's adjuvant (IFA) on days 21, 42, 52, and a final intravenous boost on day 63. Four days following the final boost the mice were euthanized, spleens removed, and splenocytes were fused with the myeloma cell line SP2/0. Fused cells were grown on hypoxanthine-aminopterin-thymidine (HAT) selective semi-solid media for 10-12 days and the resulting hybridomas clones were transferred to 96-well tissue culture plates and grown in HAT medium until the antibody titre is high. The antibody rich supernatants of the clones were isolated and tested in an ELISA assay for reactivity with C1s. Positive clones were isotyped and cultured for 32 days (post HAT selection) to identify stable expressing clones.

[0227] Hybridoma cell lines producing anti-C1s antibodies 5A1 and 5C12 were deposited with ATCC on 5/15/2013 having ATCC Accession Numbers PTA-120351 and PTA-120352.

The anti-C1s antibodies 5A1 and 5C12 were shown to bind to C1s and C1s-Pro and to neutralize biological functions of C1s in cellular and biochemical assays (*see, e.g.*, Examples 2-4).

### Example 2: Anti-C1s Antibodies Specifically Bind to C1s and C1s-Pro

[0228] First, anti-C1s antibodies were screened for C1s and C1s proenzyme binding by ELISA.

[0229] ELISA assays were conducted using standard protocols. Briefly, the assays were

conducted as follows. The day before the assay was performed, 96-well microtiter plates were coated at 0.2 µg/well of C1s-enzyme antigen in 100 µL/well carbonate coating buffer pH9.6

overnight at 4°C. Next, the plates were blocked with 3% milk powder in PBS for 1 hour at room

temperature. Next, hybridoma tissue culture supernatants were plated at 100 µL/well for 1 hour

at 37°C with shaking. The secondary antibody (1:10,000 goat anti-mouse IgG/IgM(H+L)-HRP)

was applied at 100 µL/well for 1.5 hours at room temperature with shaking. TMB substrate was

added at 50 µL/well for 5 minutes at room temperature in the dark. The reaction stopped with 50

µL/well 1M HCl and read at 450nm.

[0230] Six hybridoma supernatants (1B4, 3F8, 3G3, 5A1, 5C12, and 7C4) were tested for C1s

and C1s proenzyme binding (**FIG. 1**). All six supernatants showed strong binding signals for the

C1s proenzyme (middle column) as well as the mature C1s protease (left column). Only

background binding signals were observed with the negative control protein, human transferrin

(HT, right column) (**FIG. 1**).

[0231] These results showed that the antibodies produced by the hybridoma cells 1B4, 3F8,

3G3, 5A1, 5C12, and 7C4 specifically bind C1s and the C1s proenzyme.

#### Example 3: Anti-C1s Antibodies Inhibit Complement-Mediated Hemolysis

[0232] Next, the ability of anti-C1s antibodies to neutralize cellular activities of C1s was

tested in a complement hemolytic assay.

[0233] A modified CH50 assay (also referred to as C1F hemolysis assay) was performed that

provided limiting quantities of the C1 complex from human serum to provide greater sensitivity

for assessing C1 activity and potential C1 inhibition. In brief, the assay was conducted as

follows. First,  $3 \times 10^7$  sheep red blood cells (RBC) were incubated with anti-sheep RBC IgM

antibody to generate activated erythrocytes (EA cells). The EA cells were then incubated with

purified C4b protein to create EAC4b cells. EAC4b cells were subsequently incubated with

diluted (1:1000-1:10000) normal human serum (NHS) that was pre-incubated with or without

anti-C1s and control mouse IgG antibodies, to provide a limiting quantity of human C1. Next,

the resulting EAC14 cells were incubated with purified human C2 protein to generate EAC14b2a

cells. Finally, guinea pig serum was added in an EDTA buffer and incubated at 37°C for 30

minutes. Cell lysis was measured in a spectrophotometer at 450 nm.

[0234] Six C1s and C1s proenzyme binding antibodies (1B4, 3F8, 3G3, 5A1, 5C12, and 7C4) were tested for their ability to suppress complement mediated hemolysis. Initial testing was conducted at a single antibody concentration (165 ng/ml). Out of the six antibodies tested, only 5A1 and 5C12 antibodies showed substantial inhibition of hemolysis, whereas 1B4, 3F8, 3G3, and 7C4 were essentially inactive (**FIG. 2A**). At antibody concentrations of 165 ng/ml, 5A1 suppressed 50% of the observed hemolysis and 5C12 suppressed 90% of hemolysis observed in the hemolytic assay (**FIG. 2A**). 5A1 and 5C12 were further tested in a dose-response format; both 5A1 and 5C12 were shown to inhibit hemolysis in a dose-dependent manner (**FIG. 2B**).

[0235] These results demonstrate that not all C1s binding antibodies can neutralize complement mediated hemolysis. Two anti-C1s antibodies were identified, 5A1 and 5C12, that bind C1s and have substantial hemolysis neutralizing activity.

[0236] 5A1 and 5C12 have also been shown to inhibit complement-dependent lysis of AQP4 expressing cells incubated with anti-AQP4 antibodies. This experiment demonstrated the activity of the anti-C1s antibodies 5A1 and 5C12 in a cellular model system of NMO and their utility for the treatment of NMO. See U.S. Provisional Application No.: 61/810,222 and PCT App. No. PCT/US2014/33560.

#### Example 4: Anti-C1s Antibodies Inhibit C1s-mediated Cleavage of C4

[0237] To analyze the ability of anti-C1s antibodies to neutralize the proteolytic activity of C1s, 5A1 and 5C12 were tested for their inhibitory activity on C1s-mediated cleavage of C4.

[0238] To this end, human C1s enzyme (2 ng; Complement Technology Inc., Catalog #A103) was incubated with an approximately 10-fold molar excess (25 ng) of C1s antibodies for 30 minutes at 4°C. Protein dilutions were made in PBS containing 0.1 mg gelatin/mL. The antibody/C1s mixture was incubated with 3 mg of human C4 protein (Complement Technology Inc. Cat # A105) for 5 minutes at 37°C. SDS-DTT Sample buffer was added to each sample, mixed and immediately placed in a 37°C water bath for 15 min. The samples were loaded immediately onto a NuPage 10% BisTris SDS gel (Invitrogen Life Technologies) gel and ran for 1 hour at 150V. The gel was fixed for 1 hour, stained with Coomassie Blue for 24 hrs and de-stained overnight.

[0239] Eight anti-C1s antibodies were tested in the C4 cleavage assay. 5A1 and 5C12 inhibited C1s-mediated C4  $\alpha$ -chain cleavage if incubated at approximately 10-fold molar excess, whereas six other anti-C1s binding antibodies, including the C1s binding antibody 3F8 (see **FIG. 1**, Example 2), did not show inhibitory activity (**FIG. 3 - upper panel**). Further testing in a

dose-response format demonstrated that both 5A1 and 5C12 can inhibit C1s-mediated C4 cleavage at approximately equimolar concentrations of antibody (3ng) and C1s (2ng; **FIG. 3 - lower panel**).

#### DEPOSIT OF MATERIAL

- 5 **[0240]** The following materials have been deposited according to the Budapest Treaty in the American Type Culture Collection, ATCC Patent Depository, 10801 University Blvd., Manassas, Va. 20110-2209, USA (ATCC):

| Sample ID                                                            | Isotype     | Deposit Date | ATCC Accession No. |
|----------------------------------------------------------------------|-------------|--------------|--------------------|
| Mouse anti-C1s-RP mAb cell line<br>5A1 IgG1 producing antibody 5A1   | IgG1, kappa | 5/15/2013    | PTA-120351         |
| Mouse anti-C1s-RP mAb cell line<br>5C12 IgG1 producing antibody 5C12 | IgG1, kappa | 5/15/2013    | PTA-120352         |

- 10 **[0241]** The hybridoma cell lines producing the 5A1 and 5C12 antibodies (mouse anti-C1s-RP mAb cell line 5A1 IgG1 and mouse anti-C1s-RP mAb cell line 5C12 IgG1) have each been deposited with ATCC under conditions that assure that access to the culture will be available during pendency of the patent application and for a period of 30 years, or 5 years after the most recent request, or for the effective life of the patent, whichever is longer. A deposit will be replaced if the deposit becomes nonviable during that period. Each of the deposits is available as  
15 required by foreign patent laws in countries wherein counterparts of the subject application, or its progeny are filed. However, it should be understood that the availability of the deposits does not constitute a license to practice the subject invention in derogation of patent rights granted by governmental action.

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## CLAIMS

What is claimed is:

1. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain  
5 variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120351, or progeny thereof.
2. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain  
10 variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120351, or progeny thereof.
3. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain  
15 variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 and the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120351 or progeny thereof.
- 20 4. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120352, or progeny thereof.
- 25 5. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120352, or progeny thereof.
- 30 6. An isolated anti-C1s antibody comprising a light chain variable domain and a heavy chain variable domain, wherein the light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 and the heavy chain variable domain comprises the HVR-H1, HVR-H2,

and HVR-H3 of the monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120352, or progeny thereof.

7. The antibody of any one of claims 1-6, which specifically binds to and neutralizes a biological activity of C1s or the C1s proenzyme.
8. The antibody of any one of claims 1-7, which is a murine antibody.
9. The antibody of any one of claims 1-7, which is a humanized antibody or a chimeric antibody.
10. The antibody of any one of claims 7-9, wherein said biological activity is (1) C1s binding to C1q, (2) C1s binding to C1r, or (3) C1s binding to C2 or C4.
11. The antibody of any one of claims 7-10, wherein said biological activity is (1) the proteolytic enzyme activity of C1s, (2) the conversion of the C1s proenzyme to an active protease, (3) cleavage of C4, or (4) cleavage of C2.
12. The antibody of any one of claims 7-11, wherein said biological activity is (1) activation of the classical complement activation pathway, (2) activation of antibody and complement dependent cytotoxicity, or (3) C1F hemolysis.
13. The antibody of any one of claims 1-12, wherein said antibody is capable of neutralizing at least 30%, at least 50%, or at least 70% of C1F hemolysis.
14. An isolated murine anti-human C1s monoclonal antibody 5A1 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120351, or progeny thereof.
15. An isolated murine anti-human C1s monoclonal antibody 5C12 produced by a hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120352 or progeny thereof.
16. An isolated anti-C1s antibody which binds essentially the same C1s epitope as the antibody 5A1 produced by the hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120351.
17. An isolated anti-C1s antibody which binds essentially the same C1s epitope as the 5C12 antibody produced by the hybridoma cell line deposited with ATCC on 5/15/2013 with ATCC Accession Number PTA-120352.
18. The antibody of any one of claims 1-6, 16, and 17 which is of the IgG class.
19. The antibody of claim 18 which has an IgG<sub>1</sub>, IgG<sub>2</sub>, IgG<sub>3</sub>, or IgG<sub>4</sub> isotype.

20. The antibody of any one of claims 1-19, which is an antibody fragment.
21. The antibody of claim 20, which is a Fab fragment.
22. The antibody of claim 20, which is a F(ab')<sub>2</sub> fragment.
23. The antibody of claim 20, which is a Fab' fragment.
- 5 24. The method of any one of claims 19-23, wherein the antibody fragment specifically binds to and neutralizes a biological activity of C1s or the C1s proenzyme.
25. The method of any one of claims 19-24, wherein the antibody fragment has better brain penetration as compared to its corresponding full-length antibody.
26. The method of any one of claims 19-25, wherein the antibody fragment has a shorter half-  
10 life as compared to its corresponding full-length antibody.
27. The antibody of any one of claims 1-26, which is a bispecific antibody recognizing a first antigen and a second antigen.
28. The antibody of claim 27, wherein the first antigen is a C1s protein or a C1s proenzyme and the second antigen is an antigen facilitating transport across the blood-brain-barrier.
- 15 29. The antibody of claim 27 or 28, wherein the second antigen is selected from the group consisting of transferrin receptor (TR), insulin receptor (HIR), insulin-like growth factor receptor (IGFR), low-density lipoprotein receptor related proteins 1 and 2 (LPR-1 and 2), diphtheria toxin receptor, CRM197, a llama single domain antibody, TMEM 30(A), a protein transduction domain, TAT, Syn-B, penetratin, a poly-arginine peptide, an  
20 angiopep peptide, and ANG1005.
30. An isolated polynucleotide comprising a nucleic acid sequence encoding the antibody of any one of claims 1-29.
31. An isolated host cell comprising the nucleic acid sequence of claim 30.
32. A hybridoma cell deposited with ATCC on 5/15/2013 with ATCC Accession Number  
25 PTA-120351 or PTA-120352.
33. A pharmaceutical composition comprising the antibody of any one of claims 1-29 and a pharmaceutically acceptable carrier.
34. A method of treating or preventing an autoimmune or neurodegenerative disease in a subject in need of such treatment, the method comprising the step of administering a  
30 therapeutically effective dose of the antibody of any one of claims 1-29.
35. The method of claim 34, wherein the autoimmune disease is associated with autoantibodies activating the complement system.

36. The method of claim 34, wherein the neurodegenerative disease is associated with the loss of synapses or nerve connections.
37. The method as in any of the preceding claims, wherein the autoimmune or neurodegenerative disease is selected from the group consisting of Neuromyelitis Optica, Guillain-Barre Syndrome, Myasthenia Gravis, Bullous Pemphigoid, Alzheimer's Disease, Huntington's Disease, Parkinson's Disease, Glaucoma, Spinal Muscular Atrophy, Stroke, Traumatic Brain Injury, Multiple Sclerosis, Age-related Macular Degeneration, and normal aging.
38. The method as in any of the preceding claims, wherein the disease is associated with C1-dependent pathological synapse loss.
39. The method as in any of the preceding claims, wherein the disease is associated with synapse loss that is dependent on the complement receptor 3(CR3)/C3.
40. The method as in any of the preceding claims, wherein the disease is associated with pathological activity-dependent synaptic pruning.
41. The method as in any of the preceding claims, wherein the disease is associated with synapse phagocytosis by microglia.
42. A diagnostic kit comprising the antibody of any one of claims 1-29.
43. A method of detecting synapses in an individual having an autoimmune or neurodegenerative disease, the method comprising
- a) administering the antibody of any one of claims 1-29 to the individual, and
- b) detecting antibody bound to synapses, thereby detecting synapses in the individual.
44. The method of any one of claim 43, wherein the antibody bound to synapses is detected using imaging techniques selected from the group consisting of positron emission tomography (PET), X-ray computed tomography, single-photon emission computed tomography (SPECT), computed tomography (CT), and computed axial tomography (CAT).
45. The method of any one of claim 43 or 44, wherein the detection of antibody bound to synapses provides a quantitative measure for the number of synapses in the individual.
46. The method of any one of claims 43-45, wherein the number of synapses in the individual is measured repeatedly over a period of time and a loss of synapses in the individual is detected over time.

47. The method of claim 46, wherein the loss of synapses over time is a measure for the efficacy of a treatment for the autoimmune or neurodegenerative disease.
48. A method for detecting synapses in a biological sample, the method comprising
- 5       a) contacting the biological sample with the antibody of any one of claims 1-29, and
- b) detecting antibody bound to synapses, thereby detecting synapses in the individual.
49. The method of claim 48, further comprising obtaining the biological sample from the individual.
50. The method of claim 48 or 49, wherein the biological sample comprises a biopsy specimen, a tissue, or a cell.
- 10 51. The method of any one of claims 48-50, wherein the antibody is detected by immunofluorescence microscopy, immunocytochemistry, immunohistochemistry, ELISA, FACS analysis, or immunoprecipitation.

FIG 1

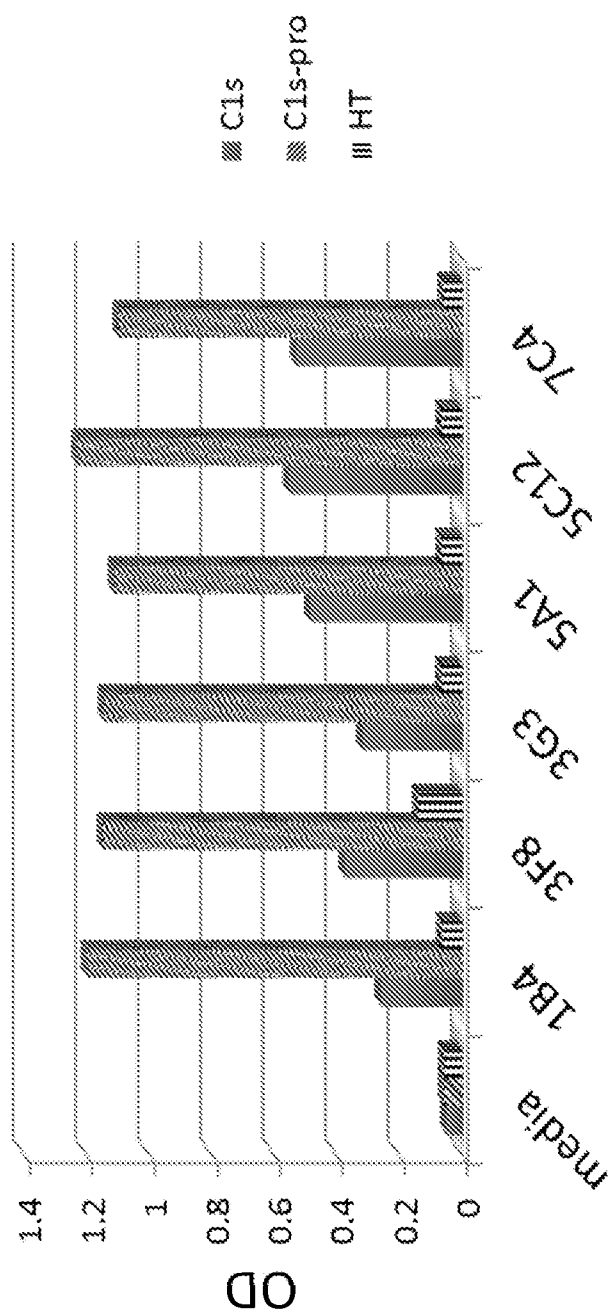


FIG 2A

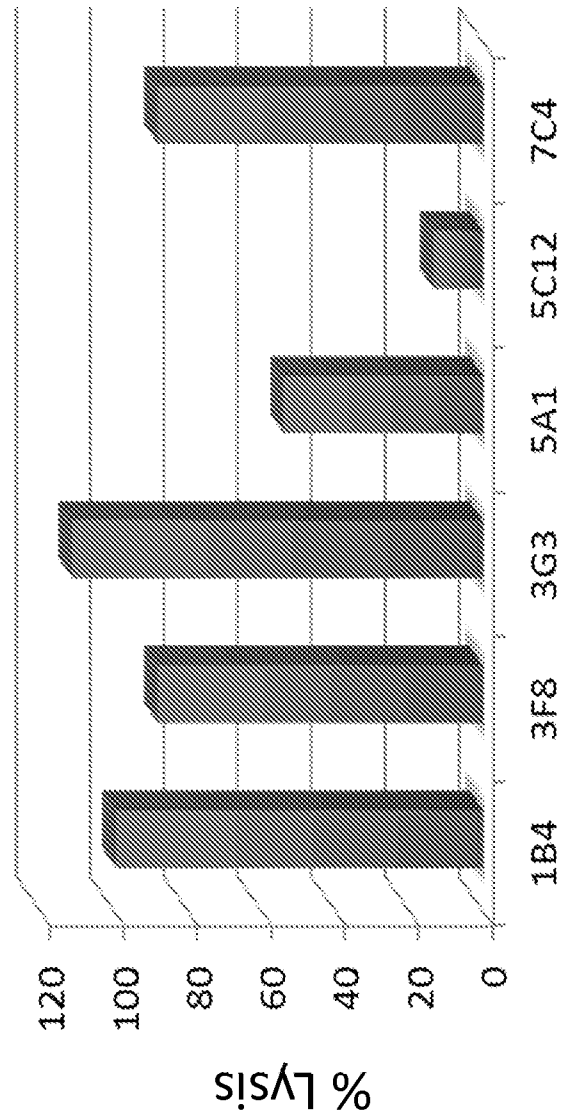


FIG 2B

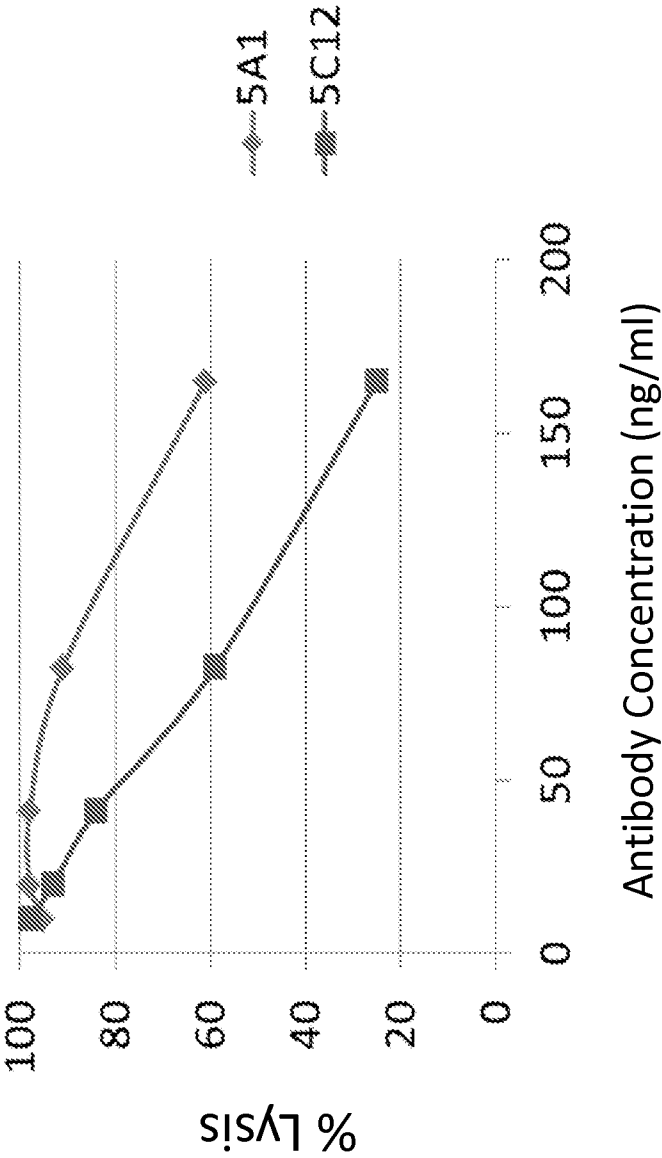


FIG 3

