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(54) Titre : TRAITEMENT DE LA DEMENCE FRONTO-TEMPORALE.

(54) Title: COMPOSITIONS AND METHODS FOR TREATING FRONTOTEMPORAL DEMENTIA

(57) **Abrégé/Abstract:**

The present disclosure relates generally to methods of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising administering to a subject an inhibitor of the complement pathway.

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(54) Title: COMPOSITIONS AND METHODS FOR TREATING FRONTOTEMPORAL DEMENTIA

(57) Abstract: The present disclosure relates generally to methods of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising administering to a subject an inhibitor of the complement pathway.

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## COMPOSITIONS AND METHODS FOR TREATING FRONTOTEMPORAL DEMENTIA

### RELATED APPLICATIONS

This Application claims the benefit of U.S. Provisional Application 62/364,013, filed  
5 July 19<sup>th</sup>, 2016, the entire contents of which are incorporated herein by reference.

### BACKGROUND

Neurodegenerative diseases are debilitating disorders of the nervous system that  
affect approximately 30 million individuals worldwide. Neurodegenerative diseases are  
challenging to treat and are also a growing health concern, both in terms of mortality and the  
10 cost of care for the afflicted. The nervous system is a fragile element of the body and has a  
limited capacity to regenerate from both acute injuries, such as stroke and spinal cord injury,  
or degenerative diseases. Neurodegenerative diseases can be characterized by progressive  
loss of neuronal subtypes in the brain and spinal cord and may be either sporadic or familial.  
Symptoms of neurodegenerative diseases commonly appear during middle or old age. Given  
15 the increasing life expectancy of the population, the incidence of these diseases will increase.  
New therapies are needed to treat neurodegenerative diseases.

Frontotemporal dementia (FTD) is a neurodegenerative disorder characterized by  
progressive deficits in either behavior and personality changes or language disturbance. FTD  
is an umbrella term for a broad spectrum of diseases including Progressive Supranuclear  
20 Palsy, Corticobasal Degeneration, and Amyotrophic Lateral Sclerosis (ALS). FTD is often  
misdiagnosed in the early stage, either as a psychiatric disorder, or as a different type of  
dementia such as Alzheimer's disease (AD). Because of the close similarity of behavioral  
changes in patients with frontotemporal dementia to those seen in patients with psychiatric  
disorders, diagnosis is challenging. Various underlying neuropathological changes lead to  
25 the FTD phenotype, all of which are characterized by selective degradation of the frontal and  
temporal cortices.

FTD is commonly used for the description of a group of early onset dementias, and is  
the second most common dementing disorder among people under 65 years of age. However,  
in 25% of the cases FTD presents in old age. The estimated prevalence of FTD is 15-  
30 22/100,000 and population studies indicate an equal gender distribution. The World Health

Organization estimates that dementia rates will double nearly every 20 years, reaching an estimated 135.5 million people in 2050.

No approved disease-modifying drugs are available for the treatment of FTD. Treatment is focused on management of behavioral symptoms. Severity of compulsion, agitation, aggressiveness, impulsivity, and aberrant eating behavior can improve with the use of selective serotonin reuptake inhibitors. Behavioral abnormalities can be managed with low doses of atypical antipsychotics. Cholinesterase inhibitors are not beneficial and can worsen behavioral abnormalities seen in patients with FTD. Memantine does not improve or delay progression of FTD symptoms.

Despite the recent discovery of two genes associated with FTD, available treatment options are not efficacious enough to prevent or treat FTD, and FTD remains difficult to eradicate completely. Thus, there is a need for new therapies to prevent, reduce the risk of developing, and treat FTD.

## SUMMARY

The present disclosure is generally directed to methods of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising administering to a subject an inhibitor of the complement pathway.

Although there are varied etiologies among neurodegenerative diseases, one cellular commonality which exists among all neurons is the synapse. Degeneration of functional synapses is of crucial importance to understanding the primary mechanisms of overall neurodegeneration. Evidence suggests that synapse loss precedes neuron loss, such as in early Alzheimer's Disease (AD). Synapse loss is inhibited by contacting neurons with inhibitors or antagonists of the complement pathway. For example, inhibitors may block activation of the complement cascade, can block the expression of specific complement proteins in neurons, interfere with signaling molecules that induce complement activation, upregulate expression of complement inhibitors in neurons, or otherwise interfere with the role of complement in synapse loss. The ability to prevent synapse loss, *e.g.* in adult brains, has important implications for maintaining normal neuronal function in a variety of neurodegenerative conditions.

Accordingly, inhibition of complement activation pathways may be a promising therapeutic strategy for preventing, reducing risk of developing, or treating frontotemporal

dementia, *e.g.*, using antibodies to inhibit the early stages of complement activation, including the complement activation pathway. Specifically, anti-C1q, anti-C1r, and anti-C1s antibodies may prevent autoantibodies from triggering the classical pathway of complement activation and prevent synapse loss resulting from the neuronal expression of complement factors.

The present disclosure is generally directed to methods of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) by inhibiting complement activation, *e.g.*, by inhibiting complement factor C1q, C1r, or C1s, *e.g.*, through the administration of antibodies, such as monoclonal, chimeric, humanized antibodies, antibody fragments, *etc.*, which bind to one or more of these complement factors.

Human complement was originally defined as the heat-labile component of plasma that “complemented” the humoral system and aided antibody-dependent killing of bacteria. Complement is now known to be a tightly regulated proteolytic network of more than 30 proteins circulating in the blood or attached to membrane surfaces that coordinate crucial roles in mammalian innate immunity, especially as it relates to inflammation and the body’s defense against invading organisms. Complement proteins are produced by many cell types and have diverse cooperative functions. For example, complement is involved in the clearance of self-antigens and apoptotic cells, forms a bridge to adaptive immunity, and also plays a significant role in tissue regeneration and tumor growth. To exercise these functions, the complement system relies on an interplay of soluble and cell-surface-bound proteins that interact with pathogen cell surfaces to mark them for destruction by phagocytes. The complement system is made up of a large number of distinct plasma proteins, primarily produced by the liver. A number of these proteins are a class of proteases known as zymogens, which are themselves activated by proteolytic cleavage. These zymogens may be widely distributed in an inactive form until an invading pathogen is detected. The complement system thus is activated through a triggered enzyme cascade.

Complement activation is initiated through three pathways: classical, alternative and lectin pathways. All three pathways are initiated by detection of surface structures by pattern recognition proteins. In addition, all three pathways merge through a common intersection, complement C3. C3 is an acute phase reactant. The liver is the main site of synthesis, although small amounts are also produced by activated monocytes and macrophages. A single chain precursor (pro-C3) of approximately 200 kD is found intracellularly; the cDNA shows that it comprises 1,663 amino acids. This is processed by proteolytic cleavage into

alpha and beta subunits, which in the mature protein are linked by disulfide bonds. Pro-C3 contains a signal peptide of 22 amino acid residues, the beta chain (645 residues) and the alpha chain (992 residues). The 2 chains are joined by 4 arginine residues that are not present in the mature protein.

5           The classical pathway is activated by the binding of the complement protein C1q directly to patches of surface-bound antibodies (IgM and IgG), and also to C-reactive protein, serum amyloid P, pentraxin 3, and other known and unknown binding sites on the surfaces of cells, synapses, and microbes.

10           C1q is a large multimeric protein of 460 kDa consisting of 18 polypeptide chains (6 C1q A chains, 6 C1q B chains, and 6 C1q C chains). C1r and C1s complement proteins bind to the C1q tail region to form the C1 complex (C1qr<sub>2</sub>s<sub>2</sub>). Binding of the C1q complex to the surface of a cell or to the complement binding domain of an antibody Fc region induces a conformational change in C1q that leads to activation of an autocatalytic enzymatic activity in C1r, which then cleaves C1s to generate an active serine protease. Once activated, C1s  
15           cleaves C4 resulting in C4b, which in turn binds to C2. C2 is cleaved by C1s, resulting in the activated form, C2a, bound to C4b and forming the C3 convertase (C4b2a) of the classical pathway. Ultimately, this pathway leads to the formation of a membrane attack complex, which lyses and kills the affected cell.

20           The lectin pathway is activated by the binding of mannose-binding lectin (MBL) through two serum serine proteases designated MASP-1 and MASP-2. Similar to the classical complement pathway, the lectin complement pathway also requires C4 and C2 for activation of C3 and other terminal components further downstream in the cascade.

25           Activation of the complement pathway generates biologically active fragments of complement proteins, *e.g.*, C3a, C4a and C5a anaphylatoxins and sC5b-9 membrane attack complex (MAC), which mediates inflammatory activities involving leukocyte chemotaxis, activation of macrophages, neutrophils, platelets, mast cells and endothelial cells, increased vascular permeability, cytolysis, and tissue injury. Antibody bound to a cell surface antigen can also activate the complement system, creating pores in the membrane of a foreign cell, or it can mediate cell destruction by antibody-dependent cell-mediated cytotoxicity (ADCC). In  
30           this process, cytotoxic cells with Fc receptors bind to the Fc region of antibodies on target cells and promote killing of the cells. Antibody bound to a foreign cell also can serve as an opsonin, enabling phagocytic cells with Fc or C3b receptors to bind and phagocytose the antibody-coated cell.

Complement is nonspecific in that it can attack both foreign invaders and host cells. Under normal conditions, host cells, including neurons, are protected from potential complement-mediated damage by various fluid-phase and membrane-bound complement regulatory proteins, such as C1 inhibitor (C1-Inh). C1-INH dissociates C1r and C1s from the active C1 complex, which protects host cells from lysis or damage from the membrane attack complex. Other proteins that protect from potential complement-mediated damage include C4b-binding protein (C4BP), factor H (FH), complement receptor 1 (CR1; CD35), complement receptor Ig (CRIg), decay accelerating factor (DAF; CD55), membrane cofactor protein (MCP; CD46), and CD59. However, deficiencies of these components or excessive activation of complement in response to certain pathological conditions can overwhelm this protective mechanism. Such unbalanced activation has been associated with a growing number of diseases and pathological disorders.

For example, various complement components are expressed by neurons and glial cells *in vitro* and *in vivo*. While their function in the brain is unknown, the expression of many of these complement proteins is upregulated by serum or inflammatory cytokines after brain injury or during the course of neurodegenerative disease pathology. Astrocytes in culture have been reported to express C1q, C1r, C1s, C4, C2, and C3, as well as the more terminal complement proteins. Neurons have been reported to express C4 and C3. C1q was shown to be expressed in neuronal synapses and to mark these synapses for elimination. *See, e.g.,* U.S. Patent Publication Nos. 2012/0195880 and 2012/328601. While selective synapse loss is an essential aspect of normal brain development (“synaptic pruning”), excessive synapse loss, especially in a mature or aging brain, results in neurodegeneration and cognitive decline. Elevated synaptic complement expression led to contribute to synaptic loss in normal aging and in neurodegenerative disease progression. Conversely, lowering complement expression was neuroprotective. Neurons affected by synapse loss may be central nervous system neurons, or peripheral nervous system neurons.

Neutralizing the activity of complement factors such as C1q, C1r, or C1s can block complement activity, prevent synapse loss, and slow neurodegenerative disease progression in disorders like FTD. Methods related to neutralizing complement factors such as C1q, C1r, or C1s in FTD are disclosed herein.

All sequences mentioned in the present disclosure are incorporated by reference from WO 2015/006504, U.S. Provisional Pat. App. No. 62/075793, U.S. Provisional Pat. App. No.

62/261376, WO 2014/186599, U.S. Pat. No. 8,877,197, each of which is hereby incorporated by reference for the antibodies and related compositions that it discloses.

In certain aspects, disclosed herein is a method of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising  
5 administering to a subject an inhibitor of the complement pathway. A variant of FTD may include a behavioral variant, a semantic variant, a non-fluent variant, and/or primary progressive aphasia. The subject may have social symptoms, emotional symptoms, eating and oral symptoms, repetitive or compulsive symptoms, sensory symptoms, motor symptoms, executive symptoms, language symptoms, neuropsychiatric symptoms, and/or other  
10 symptoms.

Disclosed herein is a method of inhibiting synapse loss in FTD, comprising administering to a patient suffering from adverse synapse loss an antibody, such as an anti-Clq antibody, an anti-Clr antibody, or an anti-Cls antibody. The method may further comprise administration of neural progenitors, or a neurogenesis enhancer. In certain  
15 preferred embodiments, the antibody binds to Clq, Clr, or Cls and inhibits complement activation.

Full-length antibodies may be prepared by the use of recombinant DNA engineering techniques. Such engineered versions include those created, for example, from natural antibody variable regions by insertions, deletions or changes in or to the amino acid  
20 sequences of the natural antibodies. Particular examples of this type include those engineered variable region domains containing at least one CDR and optionally one or more framework amino acids from one antibody and the remainder of the variable region domain from a second antibody. The DNA encoding the antibody may be prepared by deleting all but the desired portion of the DNA that encodes the full length antibody. DNA encoding chimerized  
25 antibodies may be prepared by recombining DNA substantially or exclusively encoding human constant regions and DNA encoding variable regions derived substantially or exclusively from the sequence of the variable region of a mammal other than a human. DNA encoding humanized antibodies may be prepared by recombining DNA encoding constant regions and variable regions other than the complementarity determining regions (CDRs)  
30 derived substantially or exclusively from the corresponding human antibody regions and DNA encoding CDRs derived substantially or exclusively from a mammal other than a human.

Suitable sources of DNA molecules that encode antibodies include cells, such as hybridomas, that express the full length antibody. For example, the antibody may be isolated from a host cell that expresses an expression vector that encodes the heavy and/or light chain of the antibody.

Antibody fragments may also be prepared by the use of recombinant DNA engineering techniques involving the manipulation and re-expression of DNA encoding antibody variable and constant regions. Standard molecular biology techniques may be used to modify, add or delete further amino acids or domains as desired. Any alterations to the variable or constant regions are still encompassed by the terms 'variable' and 'constant' regions as used herein. In some instances, PCR is used to generate an antibody fragment by introducing a stop codon immediately following the codon encoding the interchain cysteine of C<sub>H</sub>1, such that translation of the C<sub>H</sub>1 domain stops at the interchain cysteine. Methods for designing suitable PCR primers are well known in the art and the sequences of antibody C<sub>H</sub>1 domains are readily available. In some embodiments, stop codons may be introduced using site-directed mutagenesis techniques.

An antibody of the present disclosure may be derived from any antibody isotype ("class") including for example IgG, IgM, IgA, IgD and IgE and subclasses thereof, including for example IgG1, IgG2, IgG3 and IgG4. In certain preferred embodiments, the heavy and light chains of the antibody are from murine IgG1.

In some embodiments, the inhibitor is an antibody, such as an anti-C1q antibody, an anti-C1r antibody, or an anti-C1s antibody. The anti-C1q antibody may inhibit the interaction between C1q and an autoantibody, or between C1q and C1r, or between C1q and C1s. The anti-C1r antibody may inhibit the interaction between C1r and C1q, or between C1r and C1s. The anti-C1r antibody may inhibit the catalytic activity of C1r, or the anti-C1r antibody may inhibit the processing of pro-C1r to an active protease. The anti-C1s antibody may inhibit the interaction between C1s and C1q, or between C1s and C1r, or between C1s and C2 or C4, or the anti-C1s antibody may inhibit the catalytic activity of C1s, or it may inhibit the processing of pro-C1s to an active protease. In some instances, the anti-C1q, anti-C1r, or anti-C1s antibody causes clearance of C1q, C1r or C1s from the circulation or a tissue.

The antibody disclosed herein may be a monoclonal antibody, *e.g.*, that binds mammalian C1q, C1r, or C1s, preferably human C1q, C1r, or C1s. The antibody may be a mouse antibody, a human antibody, a humanized antibody, a chimeric antibody, or an antibody fragment. The antibodies disclosed herein may also cross the blood brain barrier

(BBB). The antibody may activate a BBB receptor-mediated transport system, such as a system that utilizes the insulin receptor, transferrin receptor, leptin receptor, LDL receptor, or IGF receptor. The antibody can be a chimeric antibody with sufficient human sequence that is suitable for administration to a human. The antibody can be glycosylated or nonglycosylated; in some embodiments, the antibody is glycosylated, *e.g.*, in a glycosylation pattern produced by post-translational modification in a CHO cell.

The antibodies of the present disclosure may also be covalently linked to a therapeutic agent, such as an anti-inflammatory protein, neurotherapeutic agent, anti-viral, anti-parasitic, anti-bacterial, endocrine drug, metabolic drug, mitotoxin, chemotherapy drug, or siRNA, for which transport across the BBB is desired. The covalent linkage between the antibody and, for example, the neurotherapeutic agent may be a linkage between any suitable portion of the antibody and the therapeutic agent, as long as it allows the antibody-agent fusion to cross the blood brain barrier and the therapeutic agent to retain a therapeutically useful portion of its activity within the central nervous system. For example, the covalent link may be between one or more light chains of the antibody and the therapeutic agent. In the case of a peptide neurotherapeutic agent (*e.g.*, a neurotrophin such as brain derived neurotrophic factor, BDNF), the peptide can be covalently linked by its carboxy or amino terminus to the carboxy or amino terminus of the light chain (LC) or heavy chain (HC) of the antibody.

Other neurotherapeutic agents that can be linked to antibodies of the present disclosure include a neurotrophin selected from brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-4/5, fibroblast growth factor (FGF)-2 and other FGFs, neurotrophin (NT)-3, erythropoietin (EPO), hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor (TGF)- $\alpha$ , TGF- $\beta$ , vascular endothelial growth factor (VEGF), interleukin-1 receptor antagonist (IL-1ra), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurturin, platelet-derived growth factor (PDGF), heregulin, neuregulin, artemin, persephin, interleukins, granulocyte-colony stimulating factor (CSF), granulocyte-macrophage-CSF, netrins, cardiotrophin-1, hedgehogs, leukemia inhibitory factor (LIF), midkine, pleiotrophin, bone morphogenetic proteins (BMPs), netrins, saposins, semaphorins, or stem cell factor (SCF).

The antibody may be a bispecific antibody, recognizing a first and a second antigen, *e.g.*, the first antigen is selected from C1q, C1r, and C1s and/or the second antigen is an antigen that allows the antibody to cross the blood-brain-barrier, such as an antigen selected

from 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  
5 peptide, or ANG1005.

An antibody of the present disclosure may bind to and inhibit a biological activity of C1q, C1r, or C1. For example, (1) C1q binding to an autoantibody, (2) C1q binding to C1r, (3) C1q binding to C1s, (4) C1q binding to phosphatidylserine, (5) C1q binding to pentraxin-3, (6) C1q binding to C-reactive protein (CRP), (7) C1q binding to globular C1q receptor  
10 (gC1qR), (8) C1q binding to complement receptor 1 (CR1), (9) C1q binding to B-amyloid, or (10) C1q binding to calreticulin. In other embodiments, the biological activity of C1q is (1) activation of the classical complement activation pathway, (2) activation of antibody and complement dependent cytotoxicity, (3) CH50 hemolysis, (4) synapse loss, (5) B-cell antibody production, (6) dendritic cell maturation, (7) T-cell proliferation, (8) cytokine  
15 production (9) microglia activation, (10) Arthus reaction, (11) phagocytosis of synapses or nerve endings or (12) activation of complement receptor 3 (CR3/C3) expressing cells.

In some embodiments, CH50 hemolysis comprises human, mouse, and/or rat CH50 hemolysis. In some embodiments, the antibody is capable of neutralizing from at least about 50%, to at least about 95% of CH50 hemolysis. The antibody may also be capable of  
20 neutralizing at least 50% of CH50 hemolysis at a dose of less than 150 ng, less than 100 ng, less than 50 ng, or less than 20 ng.

Other *in vitro* assays to measure complement activity include ELISA assays for the measurement of split products of complement components or complexes that form during complement activation. Complement activation via the classical pathway can be measured by  
25 following the levels of C4d and C4 in the serum. Activation of the alternative pathway can be measured in an ELISA by assessing the levels of Bb or C3bBbP complexes in circulation. An *in vitro* antibody-mediated complement activation assay may also be used to evaluate inhibition of C3a production.

An antibody of the present disclosure may be a monoclonal antibody, a polyclonal  
30 antibody, a recombinant antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

The antibodies of the present disclosure may also be an antibody fragment, such as a Fab fragment, a Fab' fragment, a F(ab')<sub>2</sub> fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

Disclosed herein are methods of administering to the subject a second agent, such as  
5 a second antibody or a second inhibitor. The antibody may be an anti-C1q antibody, an anti-C1r antibody, or an anti-C1s antibody. The inhibitor may be an inhibitor of antibody-dependent cellular cytotoxicity, alternative complement activation pathway; and/or an inhibitor of the interaction between the autoantibody and an autoantigen.

In some embodiments, a method is provided of determining a subject's risk of  
10 developing frontotemporal dementia, comprising: (a) administering an antibody to the subject (*i.e.* an anti-C1q, anti-C1r, or anti-C1s antibody), wherein the antibody is coupled to a detectable label; (b) detecting the detectable label to measure the amount or location of C1q, C1r, or C1s in the subject; and (c) comparing the amount or location of one or more of C1q, C1r, or C1s to a reference, wherein the risk of developing frontotemporal dementia is  
15 characterized based on a the comparison of the amount or location of one or more of C1q, C1r, or C1s to the reference. The detectable label may comprise a nucleic acid, oligonucleotide, enzyme, radioactive isotope, biotin or a fluorescent label. In some instances, the antibody may be labeled with a coenzyme such as biotin using the process of biotinylation. When biotin is used as a label, the detection of the antibody is accomplished by  
20 addition of a protein such as avidin or its bacterial counterpart streptavidin, either of which can be bound to a detectable marker such as the aforementioned dye, a fluorescent marker such as fluorescein, a radioactive isotope or an enzyme such as peroxidase. In some embodiments, the antibody is an antibody fragment (*e.g.*, Fab, Fab'-SH, Fv, scFv, or F(ab')<sub>2</sub> fragments).

25 The antibodies disclosed herein may also be coupled to a labeling group, *e.g.*, an radioisotope, radionuclide, an enzymatic group, biotinyl group, a nucleic acid, oligonucleotide, enzyme, or a fluorescent label. A labeling group may be coupled to the antibody *via* a spacer arm of any suitable length to reduce potential steric hindrance. Various methods for labeling proteins are known in the art and can be used to prepare such labeled  
30 antibodies.

Various routes of administration are contemplated. Such methods of administration include but are not limited to, topical, parenteral, subcutaneous, intraperitoneal,

intrapulmonary, intrathecal, intranasal, and intralesional administration. Parenteral infusions include intramuscular, intravenous, intraarterial, intraperitoneal, or subcutaneous administration. For treatment of central nervous system conditions, the antibody may be adapted to cross the blood-brain barrier following a non-invasive peripheral route of administration such as intravenous intramuscular, subcutaneous, intraperitoneal, or even oral administration.

The present disclosure also provides a method of detecting synapses in an individual, by a) administering an antibody from any of the embodiments to the subject, wherein the antibody is coupled to a detectable label; (b) detecting the detectable label to measure the amount or location of the antibody in the subject; and (c) comparing the amount or location of the antibody to a reference, wherein the risk of developing a disease associated with complement activation is characterized based on the comparison of the amount of antibody as compared to the reference. For example, the detectable label may comprise a nucleic acid, oligonucleotide, enzyme, radioactive isotope, biotin, or a fluorescent label (*e.g.*, fluorescein, rhodamine, cyanine dyes or BODIPY). The detectable label may be detected using an imaging agent for x-ray, CT, MRI, ultrasound, PET and SPECT.

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. All combinations of the embodiments pertaining to the invention are specifically embraced by the present invention and are disclosed herein just as if each and every combination was individually and explicitly disclosed. In addition, all sub-combinations of the various embodiments and elements thereof are also specifically embraced by the present invention and are disclosed herein just as if each and every such sub-combination was individually and explicitly disclosed herein. These and other aspects of the compositions and methods provided herein will become apparent to one of skill in the art.

The publications discussed herein are provided solely for their disclosure prior to the filing date of the present application. Nothing herein is to be construed as an admission that the present invention is not entitled to antedate such publication by virtue of prior invention. Further, the dates of publication provided can be different from the actual publication dates, which may need to be independently confirmed.

## DESCRIPTION OF THE FIGURES

**Figure 1** depicts the amino acid sequence of Homo sapiens complement C1s protein (SEQ ID NO:70).

**Figure 2** depicts an amino acid sequence of humanized IPN003 VH variant 1 (SEQ ID NO.79); and a nucleotide sequence (SEQ ID NO: 86) encoding the amino acid sequence.

**Figure 3** depicts an amino acid sequence of humanized IPN003 VH variant 2 (SEQ ID NO.80); and a nucleotide sequence (SEQ ID NO: 87) encoding the amino acid sequence.

**Figure 4** depicts an amino acid sequence of humanized IPN003 VH variant 3 (SEQ ID NO.81); and a nucleotide sequence (SEQ ID NO: 88) encoding the amino acid sequence.

**Figure 5** depicts an amino acid sequence of humanized IPN003 VH variant 4 (SEQ ID NO.82); and a nucleotide sequence (SEQ ID NO: 89) encoding the amino acid sequence.

**Figure 6** depicts an amino acid sequence of humanized IPN003 V $\kappa$  variant 1 (SEQ ID NO.83); and a nucleotide sequence (SEQ ID NO: 90) encoding the amino acid sequence.

**Figure 7** depicts an amino acid sequence of humanized IPN003 V $\kappa$  variant 2 (SEQ ID NO.84); and a nucleotide sequence (SEQ ID NO: 91) encoding the amino acid sequence.

**Figure 8** depicts an amino acid sequence of humanized IPN003 V $\kappa$  variant 3 (SEQ ID NO.85); and a nucleotide sequence (SEQ ID NO: 92) encoding the amino acid sequence.

**Figure 9** provides Table 3, which shows the amino acid differences between parental IPN003 VH and exemplary VH variants; and Table 4, which shows the amino acid differences between parental IPN003 VL and exemplary VL variants.

## DETAILED DESCRIPTION

The present disclosure relates generally to methods of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising administering to a subject an inhibitor of the complement pathway.

Suitable antibodies include antibodies that bind to complement component C1q, C1r, or C1s. Such antibodies include monoclonal antibodies and homologues, analogs, and modified or derived forms thereof, including Fab, F(ab')<sub>2</sub>, Fv and single chain antibodies.

Preferred antibodies are monoclonal antibodies, which can be raised by immunizing rodents (*e.g.*, mice, rats, hamsters and guinea pigs) with either (1) the native complement component (*e.g.*, C1q, C1r, or C1s) derived from enzymatic digestion of a purified

complement component from human plasma or serum, or (2) a recombinant complement component, or its derived fragment, expressed by either eukaryotic or prokaryotic systems. Other animals can be used for immunization, *e.g.*, non-human primates, transgenic mice expressing human immunoglobulins, and severe combined immunodeficient (SCID) mice  
5 transplanted with human B-lymphocytes.

Polyclonal and monoclonal antibodies are naturally generated as immunoglobulin (Ig) molecules in the immune system's response to a pathogen. A dominating format with a concentration of 8 mg/ml in human serum, the ~150-kDa IgG1 molecule is composed of two identical ~50-kDa heavy chains and two identical ~25-kDa light chains.

10 Hybridomas can be generated by conventional procedures by fusing B-lymphocytes from the immunized animals with myeloma cells. In addition, anti -C1q, -C1r, or -C1s antibodies can be generated by screening recombinant single-chain Fv or Fab libraries from human B-lymphocytes in a phage-display system. The specificity of the MAbs to human C1q, C1r, or C1s can be tested by enzyme linked immunosorbent assay (ELISA), Western  
15 immunoblotting, or other immunochemical techniques.

The inhibitory activity on complement activation of antibodies identified in the screening process can be assessed by hemolytic assays using either unsensitized rabbit or guinea pig RBCs for the alternative complement pathway, or sensitized chicken or sheep RBCs for the classical complement pathway. Those hybridomas that exhibit an inhibitory  
20 activity specific for the classical complement pathway are cloned by limiting dilution. The antibodies are purified for characterization for specificity to human C1q, C1r, or C1s by the assays described above.

Based on the molecular structures of the variable regions of the anti -C1q, -C1r, or -C1s antibodies, molecular modeling and rational molecular design may be used to generate  
25 and screen small molecules that mimic the molecular structures of the binding region of the antibodies and inhibit the activities of C1q, C1r, or C1s. These small molecules can be peptides, peptidomimetics, oligonucleotides, or organic compounds. The mimicking molecules can be used as inhibitors of complement activation in inflammatory indications and autoimmune diseases. Alternatively, one can use large-scale screening procedures  
30 commonly used in the field to isolate suitable small molecules from libraries of combinatorial compounds.

A suitable dosage of an antibody as disclosed herein may be between 10 and 500 µg/ml of serum. The actual dosage can be determined in clinical trials following the

conventional methodology for determining optimal dosages, i.e., administering various dosages and determining which doses provide suitable efficacy without undesirable side-effects.

Before the advent of recombinant DNA technology, proteolytic enzymes (proteases) that cleave polypeptide sequences were used to dissect the structure of antibody molecules and to determine which parts of the molecule are responsible for its various functions. Limited digestion with the protease papain cleaves antibody molecules into three fragments. Two fragments, known as Fab fragments, are identical and contain the antigen-binding activity. The Fab fragments correspond to the two identical arms of the antibody molecule, each of which consists of a complete light chain paired with the  $V_H$  and  $C_{H1}$  domains of a heavy chain. The other fragment contains no antigen binding activity but was originally observed to crystallize readily, and for this reason was named the Fc fragment (Fragment crystallizable).

A Fab molecule is an artificial ~50-kDa fragment of the Ig molecule with a heavy chain lacking constant domains  $C_{H2}$  and  $C_{H3}$ . Two heterophilic ( $V_L$ - $V_H$  and  $C_L$ - $C_{H1}$ ) domain interactions underlie the two-chain structure of the Fab molecule, which is further stabilized by a disulfide bridge between  $C_L$  and  $C_{H1}$ . Fab and IgG have identical antigen binding sites formed by six complementarity-determining regions (CDRs), three each from  $V_L$  and  $V_H$  (LCDR1, LCDR2, LCDR3 and HCDR1, HCDR2, HCDR3). The CDRs define the hypervariable antigen binding site of antibodies. The highest sequence variation is found in LCDR3 and HCDR3, which in natural immune systems are generated by the rearrangement of  $V_L$  and  $J_L$  genes or  $V_H$ ,  $D_H$  and  $J_H$  genes, respectively. LCDR3 and HCDR3 typically form the core of the antigen binding site. The conserved regions that connect and display the six CDRs are referred to as framework regions. In the three-dimensional structure of the variable domain, the framework regions form a sandwich of two opposing antiparallel  $\beta$ -sheets that are linked by hypervariable CDR loops on the outside and by a conserved disulfide bridge on the inside.

Methods are disclosed herein for protecting or treating an individual suffering from adverse effects of synapse loss, such as in frontotemporal dementia. It is shown herein that immature astrocytes in normal development produce a signal that induces neurons to express a specific complement protein, thus enabling a developmental window during which synapse elimination occurs. Expression of such a protein in development mirrors the period of

developmental synaptogenesis, being off in embryonic brain and adult brain but on at high levels in postnatal brain.

These findings have broad implications for a variety of clinical conditions, particularly neurodegenerative conditions where synapse loss is involved, such as frontotemporal dementia. Synapse loss is inhibited by contacting neurons with inhibitors or antagonists of the complement pathway. For example, inhibitors can block activation of the complement cascade, can block the expression of specific complement proteins in neurons, can interfere with signaling molecules that induce complement activation, can upregulate expression of complement inhibitors in neurons, and otherwise interfere with the role of complement in synapse loss. The ability to prevent synapse loss, *e.g.*, in adult brains, has important implications for maintaining normal neuronal function in a variety of neurodegenerative conditions.

#### *Anti-Complement C1q Antibodies*

Suitable inhibitors include an antibody that binds complement C1q protein (*i.e.*, an anti-complement C1q antibody, also referred to herein as an anti-C1q antibody and a C1q antibody) and a nucleic acid molecule that encodes such an antibody for a method of preventing, reducing risk of developing, or treating frontotemporal dementia.

All sequences mentioned in the following fourteen paragraphs are incorporated by reference from WO 2015/006504 which is hereby incorporated by reference for the antibodies and related compositions that it discloses.

Disclosed herein are methods of administering an anti-C1q antibody comprising a light chain variable domain and a heavy chain variable domain. The antibody may bind to at least human C1q, mouse C1q, or rat C1q. The antibody may be a humanized antibody, a chimeric antibody, or a human antibody. The light chain variable domain comprises the HVR-L1, HVR-L2, and HVR-L3 of the monoclonal antibody M1 produced by a hybridoma cell line deposited with Accession Number PTA-120399. The heavy chain variable domain comprises the HVR-H1, HVR-H2, and HVR-H3 of the monoclonal antibody M1 produced by a hybridoma cell line deposited with ATCC Accession Number PTA-120399.

In some embodiments, the amino acid sequence of the light chain variable domain and heavy chain variable domain comprise one or more of SEQ ID NO:5 of HVR-L1, SEQ

ID NO:6 of HVR-L2, SEQ ID NO:7 of HVR-L3, SEQ ID NO:9 of HVR-H1, SEQ ID NO:10 of HVR-H2, and SEQ ID NO:11 of HVR-H3.

The antibody may comprise a light chain variable domain amino acid sequence that is at least 85% identical to SEQ ID NO:4 and a heavy chain variable domain amino acid sequence that is at least 85% identical to SEQ ID NO:8.

Disclosed herein are methods of administering an anti-C1q antibody inhibits the interaction between C1q and an autoantibody. In some embodiments, the anti-C1q antibody inhibits the interaction between C1q and the synapse. In preferred embodiments, the anti-C1q antibody causes clearance of C1q from the circulation or tissue.

The anti-C1q antibody may bind to a C1q protein, and binds to one or more amino acids of the C1q protein within amino acid residues selected from (a) amino acid residues 196-226 of SEQ ID NO:1 (SEQ ID NO:16), or amino acid residues of a C1q protein chain A (C1qA) corresponding to amino acid residues 196-226 (GLFQVVSGGMVLQLQQGDQVWVEKDPKKGHI) of SEQ ID NO:1 (SEQ ID NO:16); (b) amino acid residues 196-221 of SEQ ID NO:1 (SEQ ID NO:17), or amino acid residues of a C1qA corresponding to amino acid residues 196-221 (GLFQVVSGGMVLQLQQGDQVWVEKDP) of SEQ ID NO:1 (SEQ ID NO:17); (c) amino acid residues 202-221 of SEQ ID NO:1 (SEQ ID NO:18), or amino acid residues of a C1qA corresponding to amino acid residues 202-221 (SGGMVLQLQQGDQVWVEKDP) of SEQ ID NO:1 (SEQ ID NO:18); (d) amino acid residues 202-219 of SEQ ID NO:1 (SEQ ID NO:19), or amino acid residues of a C1qA corresponding to amino acid residues 202-219 (SGGMVLQLQQGDQVWVEK) of SEQ ID NO:1 (SEQ ID NO:19); and (e) amino acid residues Lys 219 and/or Ser 202 of SEQ ID NO:1, or amino acid residues of a C1qA corresponding Lys 219 and/or Ser 202 of SEQ ID NO:1.

In some embodiments, the antibody further binds to one or more amino acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO:3 (SEQ ID NO:20) or amino acid residues of a C1q protein chain C (C1qC) corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of SEQ ID NO:3 (SEQ ID NO:20); (b) amino acid residues 225-240 of SEQ ID NO:3 (SEQ ID NO:21) or amino acid residues of a C1qC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO:3 (SEQ ID NO:21); (c) amino acid residues 225-232 of SEQ ID NO:3 (SEQ ID NO:22) or amino acid residues of a C1qC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO:3 (SEQ ID NO:22); (d) amino

acid residue Tyr 225 of SEQ ID NO:3 or an amino acid residue of a C1qC corresponding to amino acid residue Tyr 225 of SEQ ID NO:3; (e) amino acid residues 174-196 of SEQ ID NO:3 (SEQ ID NO:23) or amino acid residues of a C1qC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKVVTFCGHT) of SEQ ID NO:3 (SEQ ID NO:23);

5 (f) amino acid residues 184-192 of SEQ ID NO:3 (SEQ ID NO:24) or amino acid residues of a C1qC corresponding to amino acid residues 184-192 (RSGVKVVTFF) of SEQ ID NO:3 (SEQ ID NO:24); (g) amino acid residues 185-187 of SEQ ID NO:3 or amino acid residues of a C1qC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO:3; (h) amino acid residue Ser 185 of SEQ ID NO:3 or an amino acid residue of a C1qC corresponding to

10 amino acid residue Ser 185 of SEQ ID NO:3.

In certain embodiments, the anti-C1q antibody binds to amino acid residue Lys 219 and Ser 202 of the human C1qA as shown in SEQ ID NO:1 or amino acids of a human C1qA corresponding to Lys 219 and Ser 202 as shown in SEQ ID NO:1, and amino acid residue Tyr 225 of the human C1qC as shown in SEQ ID NO:3 or an amino acid residue of a human

15 C1qC corresponding to Tyr 225 as shown in SEQ ID NO:3. In certain embodiments, the anti-C1q antibody binds to amino acid residue Lys 219 of the human C1qA as shown in SEQ ID NO:1 or an amino acid residue of a human C1qA corresponding to Lys 219 as shown in SEQ ID NO:1, and amino acid residue Ser 185 of the human C1qC as shown in SEQ ID NO:3 or an amino acid residue of a human C1qC corresponding to Ser 185 as shown in SEQ

20 ID NO:3.

In some embodiments, the anti-C1q antibody binds to a C1q protein and binds to one or more amino acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO:3 (SEQ ID NO:20) or amino acid residues of a C1qC corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of

25 SEQ ID NO:3 (SEQ ID NO:20); (b) amino acid residues 225-240 of SEQ ID NO:3 (SEQ ID NO:21) or amino acid residues of a C1qC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO:3 (SEQ ID NO:21); (c) amino acid residues 225-232 of SEQ ID NO:3 (SEQ ID NO:22) or amino acid residues of a C1qC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO:3 (SEQ ID NO:22); (d) amino

30 acid residue Tyr 225 of SEQ ID NO:3 or an amino acid residue of a C1qC corresponding to amino acid residue Tyr 225 of SEQ ID NO:3; (e) amino acid residues 174-196 of SEQ ID NO:3 (SEQ ID NO:23) or amino acid residues of a C1qC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKVVTFCGHT) of SEQ ID NO:3 (SEQ ID NO:23);

(f) amino acid residues 184-192 of SEQ ID NO:3 (SEQ ID NO:24) or amino acid residues of a C1qC corresponding to amino acid residues 184-192 (RSGVKVVTFF) of SEQ ID NO:3 (SEQ ID NO:24); (g) amino acid residues 185-187 of SEQ ID NO:3 or amino acid residues of a C1qC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO:3; (h) amino acid residue Ser 185 of SEQ ID NO:3 or an amino acid residue of a C1qC corresponding to amino acid residue Ser 185 of SEQ ID NO:3.

In some embodiments, the anti-C1q antibody of this disclosure inhibits the interaction between C1q and C1s. In some embodiments, the anti-C1q antibody inhibits the interaction between C1q and C1r. In some embodiments the anti-C1q antibody inhibits the interaction between C1q and C1s and between C1q and C1r. In some embodiments, the anti-C1q antibody inhibits the interaction between C1q and another antibody, such as an autoantibody. In preferred embodiments, the anti-C1q antibody causes clearance of C1q from the circulation or tissue. In some embodiments, the anti-C1q antibody inhibits the 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 C1q antibody inhibits an interaction, such as the C1q-C1s interaction, at approximately equimolar concentrations of C1q and the anti-C1q antibody. In other embodiments, the anti-C1q antibody binds to C1q 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-C1q antibody binds C1q with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than 1.0:1. In certain embodiments, the anti-C1q antibody binds C1q with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than 1.0:1. In certain embodiments, the anti-C1q antibody binds C1q with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than 1.0:1. In some embodiments, the anti-C1q antibody inhibits the interaction between C1q and C1r, or between C1q and C1s, or between C1q and both C1r and C1s. In some embodiments, the anti-C1q antibody inhibits the interaction between C1q and C1r, between C1q and C1s, and/or between C1q and both C1r and C1s. In some embodiments, the anti-C1q antibody binds to the C1q A-chain. In other embodiments, the anti-C1q antibody binds to the C1q B-

chain. In other embodiments, the anti-C1q antibody binds to the C1q C-chain. In some  
embodiments, the anti-C1q antibody binds to the C1q A-chain, the C1q B-chain and/or the  
C1q C-chain. In some embodiments, the anti-C1q antibody binds to the globular domain of  
the C1q A-chain, B-chain, and/or C-chain. In other embodiments, the anti-C1q antibody  
5 binds to the collagen-like domain of the C1q A-chain, the C1q B-chain, and/or the C1q C-  
chain.

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 C1q  
and C1r, the interaction occurring in the presence of the antibody may be reduced by at least  
10 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% relative to a control wherein the antibodies of  
this disclosure are absent. 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% relative  
to a control wherein the antibodies of this disclosure are absent.

15 In some embodiments, the antibodies of this disclosure inhibit C2 or 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%, or 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 or C4-cleavage are well known in the art. The EC<sub>50</sub> values for  
20 antibodies of this disclosure with respect C2 or C4-cleavage may be less than 3 µg/ml; 2.5  
µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. In  
some embodiments, the antibodies of this disclosure inhibit C2 or C4-cleavage at  
approximately equimolar concentrations of C1q and the respective anti-C1q antibody.

In some embodiments, the antibodies of this disclosure inhibit autoantibody-  
25 dependent and complement-dependent cytotoxicity (CDC) 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%, or 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. The EC<sub>50</sub> values for antibodies  
of this disclosure with respect to inhibition of autoantibody-dependent and complement-  
30 dependent cytotoxicity may be less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml;  
0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml.

In some embodiments, the antibodies of this disclosure inhibit complement-dependent cell-mediated cytotoxicity (CDCC) 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%, or 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 CDCC are well known in the art. The EC<sub>50</sub> values for antibodies of this disclosure with respect CDCC inhibition may be 1 less than 3 µg/ml; 2.5 µg/ml; 2.0 µg/ml; 1.5 µg/ml; 1.0 µg/ml; 0.5 µg/ml; 0.25 µg/ml; 0.1 µg/ml; 0.05 µg/ml. In some embodiments, the antibodies of this disclosure inhibit CDCC but not antibody-dependent cellular cytotoxicity (ADCC).

#### *Humanized anti-complement C1q Antibodies*

Humanized antibodies of the present disclosure specifically bind to a complement factor C1q and/or C1q protein in the C1 complex of the classical complement pathway. The humanized anti-C1q antibody may specifically bind to human C1q, human and mouse C1q, to rat C1q, or human C1q, mouse C1q, and rat C1q.

All sequences mentioned in the following sixteen paragraphs are incorporated by reference from U.S. Provisional Pat. App. No. 62/075793, which is hereby incorporated by reference for the antibodies and related compositions that it discloses.

In some embodiments, the human heavy chain constant region is a human IgG4 heavy chain constant region comprising the amino acid sequence of SEQ ID NO:47, or with at least 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90% homology to SEQ ID NO: 37. The human IgG4 heavy chain constant region may comprise an Fc region with one or more modifications and/or amino acid substitutions according to Kabat numbering. In such cases, the Fc region comprises a leucine to glutamate amino acid substitution at position 248, wherein such a substitution inhibits the Fc region from interacting with an Fc receptor. In some embodiments, the Fc region comprises a serine to proline amino acid substitution at position 241, wherein such a substitution prevents arm switching in the antibody.

The amino acid sequence of human IgG4 (S241P L248E) heavy chain constant domain is:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ  
SSGLYSLSSVVTVPSSSLGTKTYTCNVDPKPSNTKVDKRVERSKYGPCCPPCPAPEFEG

GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE  
EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYT  
LPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS  
RLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLGLGK (SEQ ID NO: 47).

5 The antibody may comprise a heavy chain variable domain and a light chain variable domain, wherein the heavy chain variable domain comprises an amino acid sequence selected from any one of SEQ ID NOs: 31-34, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from any one of SEQ ID NOs: 31-34. In certain such embodiments, the light chain variable domain comprises an amino acid sequence  
10 selected from any one of SEQ ID NOs: 35-38, or an amino acid sequence with at least about 90% homology to the amino acid sequence selected from any one of SEQ ID NOs: 35-38.

The amino acid sequence of heavy chain variable domain variant 1 (VH1) is:  
QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHPN**  
**SGSINYNEKFES**KATITVDKSTSTAYMQLSSLTSEDSAVYYCAG**ERDSTEVLPM****MDY**  
15 WGQGTSVTVSS (SEQ ID NO: 31). The hyper variable regions (HVRs) of VH1 are depicted in bolded and underlined text.

The amino acid sequence of heavy chain variable domain variant 2 (VH2) is:  
QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHPN**  
**SGSINYNEKFES**RATITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM****MDY**  
20 WGQGTTVTVSS (SEQ ID NO: 32). The hyper variable regions (HVRs) of VH2 are depicted in bolded and underlined text.

The amino acid sequence of heavy chain variable domain variant 3 (VH3) is:  
QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVKQAPGQGLEWIG**VIHPN**  
**SGSINYNEKFES**RVTITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM****MDY**  
25 WGQGTTVTVSS (SEQ ID NO: 33). The hyper variable regions (HVRs) of VH3 are depicted in bolded and underlined text.

The amino acid sequence of heavy chain variable domain variant 4 (VH4) is:  
QVQLVQSGAELKKPGASVKVSCKSS**GYHFTSYWMH**WVRQAPGQGLEWIG**VIHPN**  
**SGSINYNEKFES**RVTITVDKSTSTAYMELSSLRSEDTAVYYCAG**ERDSTEVLPM****MDY**  
30 WGQGTTVTVSS (SEQ ID NO: 34). The hyper variable regions (HVRs) of VH4 are depicted in bolded and underlined text.

The amino acid sequence of kappa light chain variable domain variant 1 (V $\kappa$ 1) is:  
 DVQITQSPSYLAASLGERATINC**RASKSINKYLA**WYQQKPGKTNKLLIY**SGSTLQSGI**  
 PARFSGSGSGTDFTLTISSELEPEDFAMYYC**QQHNEYPLT**FGQGGTKLEIK (SEQ ID NO:  
 35). The hyper variable regions (HVRs) of V $\kappa$ 1 are depicted in bolded and underlined text.

5 The amino acid sequence of kappa light chain variable domain variant 2 (V $\kappa$ 2) is:  
 DVQITQSPSSLSASLGERATINC**RASKSINKYLA**WYQQKPGKANKLLIY**SGSTLQSGI**  
 PARFSGSGSGTDFTLTISSELEPEDFAMYYC**QQHNEYPLT**FGQGGTKLEIK (SEQ ID NO:  
 36). The hyper variable regions (HVRs) of V $\kappa$ 2 are depicted in bolded and underlined text.

The amino acid sequence of kappa light chain variable domain variant 3 (V $\kappa$ 3) is:  
 10 DVQITQSPSSLSASLGERATINC**RASKSINKYLA**WYQQKPGKAPKLLIY**SGSTLQSGI**  
 PARFSGSGSGTDFTLTISSELEPEDFAMYYC**QQHNEYPLT**FGQGGTKLEIK (SEQ ID NO:  
 37). The hyper variable regions (HVRs) of V $\kappa$ 3 are depicted in bolded and underlined text.

The amino acid sequence of kappa light chain variable domain variant 4 (V $\kappa$ 4) is:  
 DIQLTQSPSSLSASLGERATINC**RASKSINKYLA**WYQQKPGKAPKLLIY**SGSTLQSGIP**  
 15 ARFSGSGSGTDFTLTISSELEPEDFAMYYC**QQHNEYPLT**FGQGGTKLEIK (SEQ ID NO:  
 38). The hyper variable regions (HVRs) of V $\kappa$ 4 are depicted in bolded and underlined text.

In some embodiments, humanized anti-C1q antibodies of the present disclosure include a heavy chain variable region that contains an Fab region and a heavy chain constant regions that contains an Fc region, where the Fab region specifically binds to a C1q protein  
 20 of the present disclosure, but the Fc region is incapable of binding the C1q protein. In some embodiments, the Fc region is from a human IgG1, IgG2, IgG3, or IgG4 isotype. In some embodiments, the Fc region is incapable of inducing complement activity and/or incapable of inducing antibody-dependent cellular cytotoxicity (ADCC). In some embodiments, the Fc region comprises one or more modifications, including, without limitation, amino acid  
 25 substitutions. In certain embodiments, the Fc region of humanized anti-C1q antibodies of the present disclosure comprise an amino acid substitution at position 248 according to Kabat numbering convention or a position corresponding to position 248 according to Kabat numbering convention, and/or at position 241 according to Kabat numbering convention or a position corresponding to position 241 according to Kabat numbering convention. In some  
 30 embodiments, the amino acid substitution at position 248 or a positions corresponding to position 248 inhibits the Fc region from interacting with an Fc receptor. In some

embodiments, the amino acid substitution at position 248 or a positions corresponding to position 248 is a leucine to glutamate amino acid substitution. In some embodiments, the amino acid substitution at position 241 or a positions corresponding to position 241 prevents arm switching in the antibody. In some embodiments, the amino acid substitution at position 241 or a positions corresponding to position 241 is a serine to proline amino acid substitution. In certain embodiments, the Fc region of humanized anti-C1q antibodies of the present disclosure comprises the amino acid sequence of SEQ ID NO: 37, or an amino acid sequence with at least about 70%, at least about 75%, at least about 80% at least about 85% at least about 90%, or at least about 95% homology to the amino acid sequence of SEQ ID NO: 47.

In some embodiments, humanized anti-C1q antibodies of the present disclosure may bind to a C1q protein and binds to one or more amino acids of the C1q protein within amino acid residues selected from (a) amino acid residues 196-226 of SEQ ID NO: 39 (SEQ ID NO:42), or amino acid residues of a C1q protein chain A (C1qA) corresponding to amino acid residues 196-226 (GLFQVVSGGMVLQLQQGDQVWVEKDPKKGHI) of SEQ ID NO: 39 (SEQ ID NO:42); (b) amino acid residues 196-221 of SEQ ID NO: 39 (SEQ ID NO:43), or amino acid residues of a C1qA corresponding to amino acid residues 196-221 (GLFQVVSGGMVLQLQQGDQVWVEKDP) of SEQ ID. NO: 39 (SEQ ID NO:43); (c) amino acid residues 202-221 of SEQ ID NO:39 (SEQ ID NO:44), or amino acid residues of a C1qA corresponding to amino acid residues 202-221 (SGGMVLQLQQGDQVWVEKDP) of SEQ ID NO: 39 (SEQ ID NO:44); (d) amino acid residues 202-219 of SEQ ID NO: 39 (SEQ ID NO:45), or amino acid residues of a C1qA corresponding to amino acid residues 202-219 (SGGMVLQLQQGDQVWVEK) of SEQ ID NO: 39 (SEQ ID NO:45); and (e) amino acid residues Lys 219 and/or Ser 202 of SEQ ID NO: 39, or amino acid residues of a C1qA corresponding Lys 219 and/or Ser 202 of SEQ ID NO: 39.

In some embodiments, the humanized anti-C1q antibodies may further binds to one or more amino acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO: 41 (SEQ ID NO:46) or amino acid residues of a C1q protein chain C (C1qC) corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of SEQ ID NO: 41 (SEQ ID NO:46); (b) amino acid residues 225-240 of SEQ ID NO: 41 (SEQ ID NO:48) or amino acid residues of a C1qC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO: 41 (SEQ ID NO:48); (c) amino acid residues 225-232 of SEQ ID NO: 41 (SEQ ID NO:49) or

amino acid residues of a C1qC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO: 41 (SEQ ID NO:49); (d) amino acid residue Tyr 225 of SEQ ID NO: 41 or an amino acid residue of a C1qC corresponding to amino acid residue Tyr 225 of SEQ ID NO: 41; (e) amino acid residues 174-196 of SEQ ID NO: 41 (SEQ ID NO:50) or amino acid residues of a C1qC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKVVTFCGHT) of SEQ ID NO: 41 (SEQ ID NO:50); (f) amino acid residues 184-192 of SEQ ID NO: 41 (SEQ ID NO:30) or amino acid residues of a C1qC corresponding to amino acid residues 184-192 (RSGVKVVTFC) of SEQ ID NO: 41 (SEQ ID NO:30); (g) amino acid residues 185-187 of SEQ ID NO: 41 or amino acid residues of a C1qC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO: 41; (h) amino acid residue Ser 185 of SEQ ID NO: 41 or an amino acid residue of a C1qC corresponding to amino acid residue Ser 185 of SEQ ID NO: 41.

In certain embodiments, humanized anti-C1q antibodies of the present disclosure may bind to amino acid residue Lys 219 and Ser 202 of the human C1qA as shown in SEQ ID NO: 39 or amino acids of a human C1qA corresponding to Lys 219 and Ser 202 as shown in SEQ ID NO: 39, and amino acid residue Tyr 225 of the human C1qC as shown in SEQ ID NO: 41 or an amino acid residue of a human C1qC corresponding to Tyr 225 as shown in SEQ ID NO: 41. In certain embodiments, the anti-C1q antibody binds to amino acid residue Lys 219 of the human C1qA as shown in SEQ ID NO: 39 or an amino acid residue of a human C1qA corresponding to Lys 219 as shown in SEQ ID NO: 39, and amino acid residue Ser 185 of the human C1qC as shown in SEQ ID NO: 41 or an amino acid residue of a human C1qC corresponding to Ser 185 as shown in SEQ ID NO: 41.

In some embodiments, humanized anti-C1q antibodies of the present disclosure may bind to a C1q protein and binds to one or more amino acids of the C1q protein within amino acid residues selected from: (a) amino acid residues 218-240 of SEQ ID NO: 41 (SEQ ID NO:46) or amino acid residues of a C1qC corresponding to amino acid residues 218-240 (WLAVNDYYDMVGI QGSDSVFSGF) of SEQ ID NO: 41 (SEQ ID NO:46); (b) amino acid residues 225-240 of SEQ ID NO: 41 (SEQ ID NO:48) or amino acid residues of a C1qC corresponding to amino acid residues 225-240 (YDMVGI QGSDSVFSGF) of SEQ ID NO: 41 (SEQ ID NO:48); (c) amino acid residues 225-232 of SEQ ID NO: 41 (SEQ ID NO:49) or amino acid residues of a C1qC corresponding to amino acid residues 225-232 (YDMVGIQG) of SEQ ID NO: 41 (SEQ ID NO:49); (d) amino acid residue Tyr 225 of SEQ ID NO: 41 or an amino acid residue of a C1qC corresponding to amino acid residue Tyr 225 of SEQ ID NO:

41; (e) amino acid residues 174-196 of SEQ ID NO: 41 (SEQ ID NO:50) or amino acid residues of a C1qC corresponding to amino acid residues 174-196 (HTANLCVLLYRSGVKVVTF CGHT) of SEQ ID NO: 41 (SEQ ID NO:50); (f) amino acid residues 184-192 of SEQ ID NO: 41 (SEQ ID NO:50) or amino acid residues of a C1qC corresponding to amino acid residues 184-192 (RSGVKVVTF) of SEQ ID NO: 41 (SEQ ID NO:50); (g) amino acid residues 185-187 of SEQ ID NO: 41 or amino acid residues of a C1qC corresponding to amino acid residues 185-187 (SGV) of SEQ ID NO: 41; (h) amino acid residue Ser 185 of SEQ ID NO: 41 or an amino acid residue of a C1qC corresponding to amino acid residue Ser 185 of SEQ ID NO: 41.

### *Anti-Complement C1s Antibodies*

Suitable inhibitors include an antibody that binds complement C1s protein (i.e., an anti-complement C1s antibody, also referred to herein as an anti-C1s antibody and a C1s antibody) and a nucleic acid molecule that encodes such an antibody. Complement C1s is an attractive target as it is upstream in the complement cascade and has a narrow range of substrate specificity. Furthermore it is possible to obtain antibodies (for example, but not limited to, monoclonal antibodies) that specifically bind the activated form of C1s.

All sequences mentioned in the following two paragraphs are incorporated by reference from WO 2014/186599, which is hereby incorporated by reference for the antibodies and related compositions that it discloses.

In certain aspects, disclosed herein are methods of administering an anti-C1s antibody. The antibody may be a murine, humanized, or chimeric antibody. In some embodiments, the light chain variable domain comprises HVR-L1, HVR-L2, and HVR-L3, and the heavy chain comprises HVR-H1, HVR-H2, and HVR-H3 of a murine anti-human C1s 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 embodiments, 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 murine anti-human C1s 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 some embodiments, antibodies specifically bind to and inhibit a biological activity of C1s or the C1s proenzyme, such as C1s binding to C1q, C1s binding to C1r, or C1s

binding to C2 or C4. The biological activity may be a proteolytic enzyme activity of C1s, the conversion of the C1s proenzyme to an active protease, or proteolytic cleavage of C2 or C4. In certain embodiments, the biological activity is activation of the classical complement activation pathway, activation of antibody and complement dependent cytotoxicity, or C1F  
5 hemolysis.

All sequences in the following sixty-two paragraphs are incorporated by reference from Van Vlasselaer, U.S. Pat. No. 8,877,197, which is hereby incorporated by reference for the antibodies and related compositions that it discloses.

Disclosed herein are methods of administering a humanized monoclonal antibody  
10 that specifically binds an epitope within a region encompassing domains IV and V of complement component C1s. In some cases, the antibody inhibits binding of C1s to complement component 4 (C4) and/or does not inhibit protease activity of C1s. In some embodiments, the method comprises administering a humanized monoclonal antibody that binds complement component C1s in a C1 complex with high avidity.

15 Disclosed herein are methods of administering an anti-C1s antibody with one or more of the complementarity determining regions (CDRs) of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:57 and/or one or more of the CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:58. The anti-C1s antibody may bind a human or rat complement C1s protein. In some  
20 embodiments, an anti-C1s antibody inhibits cleavage of at least one substrate cleaved by complement C1s protein.

In certain embodiments, the antibody comprises: a) a complementarity determining region (CDR) having an amino acid sequence selected from SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56; and/ or b) a CDR  
25 having an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:53, SEQ ID NO:64, SEQ ID NO:65: and SEQ ID NO:66.

The antibody may comprise a CDR-L1 having amino acid sequence SEQ ID NO:51, a CDR-L2 having amino acid sequence SEQ ID NO:52, a CDR-L3 having amino acid sequence SEQ ID NO:53, a CDR-H1 having amino acid sequence SEQ ID NO:54, a CDR-  
30 H2 having amino acid sequence SEQ ID NO:55, and a CDR-H3 having amino acid sequence SEQ ID NO:56.

In other embodiments, the antibody may comprise light chain CDRs of a variable region with an amino acid sequence of SEQ ID NO:67, and/or heavy chain CDRs of a variable region with an amino acid sequence of SEQ ID NO:68.

The antibody can be a humanized antibody that specifically binds complement component C1s, wherein the antibody competes for binding the epitope with an antibody that comprises one or more of the CDRs of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:57 or SEQ ID NO:67, and/or one or more of the CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:58 or SEQ ID NO:68.

In other instances, the antibody can be a humanized antibody that specifically binds complement C1s, wherein the antibody is selected from: a) a humanized antibody that specifically binds an epitope within the complement C1s protein, wherein the antibody competes for binding the epitope with an antibody that comprises a CDR having an amino acid sequence selected from SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56; and b) a humanized antibody that specifically binds an epitope within the complement C1s protein, wherein the antibody competes for binding the epitope with an antibody that comprises a CDR having an amino acid sequence selected from SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:53, SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66. In some cases, the antibody competes for binding the epitope with an antibody that comprises heavy and light chain CDRs comprising: a) SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:69, SEQ ID NO:55, and SEQ ID NO:56; or b) SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:53, SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66.

The antibody may comprise a light chain region and a heavy chain region that are present in separate polypeptides. The antibody may comprise an Fc region.

Disclosed herein is an anti-C1s antibody comprising a light chain variable region of an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:57, and a heavy chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:58.

The anti-C1s antibody may be selected from an antigen binding fragment, Ig monomer, a Fab fragment, a F(ab')<sub>2</sub> fragment, a Fd fragment, a scFv, a scAb, a dAb, a Fv, a single domain heavy chain antibody, a single domain light chain antibody, a mono-specific antibody, a bi-specific antibody, or a multi-specific antibody.

Disclosed herein are methods of administering an antibody that competes for binding the epitope bound by antibody IPN003 (also referred to herein as “IPN-M34” or “M34” or “TNT003”), *e.g.*, an antibody comprising a variable domain of antibody IPN003, such as antibody IPN003.

5 In some embodiments, the method comprises administering an antibody that specifically binds an epitope within a complement C1s protein. In some embodiments, the isolated anti-C1s antibody binds an activated C1s protein. In some embodiments, the isolated anti-C1s antibody binds an inactive form of C1s. In other instances, the isolated anti-C1s antibody binds both an activated C1s protein and an inactive form of C1s.

10 In some embodiments, the method comprises administering a monoclonal antibody that inhibits cleavage of C4, where the isolated monoclonal antibody does not inhibit cleavage of C2. In some embodiments, the method comprises administering a monoclonal antibody that inhibits cleavage of C2, where the isolated monoclonal antibody does not inhibit cleavage of C4. In some cases, the isolated monoclonal antibody is humanized. In  
15 some cases, the antibody inhibits a component of the classical complement pathway. In some cases, the component of the classical complement pathway that is inhibited by the antibody is C1s. The present disclosure also provides methods of treating a complement-mediated disease or disorder, by administering to an individual in need thereof an isolated monoclonal antibody that inhibits cleavage of C4, or a pharmaceutical composition comprising the  
20 isolated monoclonal antibody, where the isolated monoclonal antibody does not inhibit cleavage of C2.

In some embodiments, the method comprises administering a monoclonal antibody that inhibits cleavage of C2 or C4 by C1s, *i.e.*, inhibits C1s-mediated proteolytic cleavage of C2 or C4. In some cases, the monoclonal antibody is humanized. In some cases, the antibody  
25 inhibits cleavage of C2 or C4 by C1s by inhibiting binding of C2 or C4 to C1s; for example, in some cases, the antibody inhibits C1s-mediated cleavage of C2 or C4 by inhibiting binding of C2 or C4 to a C2 or C4 binding site of C1s. Thus, in some cases, the antibody functions as a competitive inhibitor. The present disclosure also provides methods of treating frontotemporal dementia, by administering to an individual in need thereof an isolated  
30 monoclonal antibody that inhibits cleavage of C2 or C4 by C1s, *i.e.*, inhibits C1s-mediated proteolytic cleavage of C2 or C4.

In some embodiments, the method comprises administering a monoclonal antibody that inhibits cleavage of C4 by C1s, where the antibody does not inhibit cleavage of

complement component C2 by C1s; i.e., the antibody inhibits C1s-mediated cleavage of C4, but does not inhibit C1s-mediated cleavage of C2. In some cases, the monoclonal antibody is humanized. In some cases, the monoclonal antibody inhibits binding of C4 to C1s, but does not inhibit binding of C2 to C1s. In some embodiments, the method comprises treating a complement-mediated disease or disorder, by administering to an individual in need thereof an isolated monoclonal antibody that inhibits cleavage of C4 by C1s, where the antibody does not inhibit cleavage of complement component C2 by C1s; i.e., the antibody inhibits C1s-mediated cleavage of C4, but does not inhibit C1s-mediated cleavage of C2. In some embodiments of the method, the antibody is humanized.

In some embodiments, the method comprises administering a humanized monoclonal antibody that specifically binds an epitope within a region encompassing domains IV and V of C1s. For example, the humanized monoclonal antibody specifically binds an epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70. In some cases, the humanized monoclonal antibody specifically binds an epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70, and inhibits binding of C4 to C1s. In some embodiments, the method comprises treating a complement-mediated disease or disorder, by administering to an individual in need thereof a humanized monoclonal antibody that specifically binds an epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70, and inhibits binding of C4 to C1s.

In some embodiments, the method comprises administering a humanized monoclonal antibody that specifically binds a conformational epitope within a region encompassing domains IV and V of C1s. For example, the humanized monoclonal antibody that specifically binds a conformational epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70. In some cases, the humanized monoclonal antibody specifically binds a conformational epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70, and inhibits binding of C4 to C1s. In some embodiments, the method comprises frontotemporal dementia, the method comprising administering to an individual in need thereof a humanized monoclonal antibody that specifically binds a conformational epitope within amino acids 272-422 of the amino acid sequence depicted in FIG. 1 and set forth in SEQ ID NO:70, and inhibits binding of C4 to C1s.

In some embodiments, the method comprises administering a monoclonal antibody that binds complement component C1s in a C1 complex. The C1 complex is composed of 6 molecules of C1q, 2 molecules of C1r, and 2 molecules of C1s. In some cases, the monoclonal antibody is humanized. Thus, in some cases, the humanized monoclonal antibody that binds complement component C1s in a C1 complex. In some cases, the antibody binds C1s present in a C1 complex with high avidity.

In some embodiments, the anti-C1s antibody (*e.g.*, a subject antibody that specifically binds an epitope in a complement C1s protein) comprises: a) a light chain region comprising one, two, or three VL CDRs of an IPN003 antibody; and b) a heavy chain region comprising one, two, or three VH CDRs of an IPN003 antibody; where the VH and VL CDRs are as defined by Kabat (see, *e.g.*, Table 1; and Kabat 1991).

In other embodiments, the anti-C1s antibody (*e.g.*, a subject antibody that specifically binds an epitope in a complement C1s protein) comprises: a) a light chain region comprising one, two, or three VL CDRs of an IPN003 antibody; and b) a heavy chain region comprising one, two, or three VH CDRs of an IPN003 antibody; where the VH and VL CDRs are as defined by Chothia (see, *e.g.*, Table 1, and Chothia 1987).

CDR amino acid sequences, and VL and VH amino acid sequences, of IPN003 antibody are provided in Table 2. Table 2 also provides the SEQ ID NOs assigned to each of the amino acid sequences.

In some embodiments, the anti-C1s antibody (*e.g.*, a subject antibody that specifically binds an epitope in a complement C1s protein) comprises: a) a light chain region comprising one, two, or three CDRs selected from SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53; and b) a heavy chain region comprising one, two, or three CDRs selected from SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56. In some of these embodiments, the anti-C1s antibody includes a humanized VH and/or VL framework region.

SEQ ID NO. 51: SSVSSSYLHWYQ;

SEQ ID NO. 52: STSNLASGVP;

SEQ ID NO. 53: HQYYRLPPIT;

SEQ ID NO. 54: GFTFSNYAMSWV;

SEQ ID NO. 55: ISSGGSHITYY;

SEQ ID NO. 56: ARLFTGYAMDY.

In some embodiments, the anti-C1s antibody comprises a CDR having an amino acid sequence selected from SEQ ID NO:51, SEQ ID NO:52, SEQ ID NO:53, SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

5 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising amino acid sequences SEQ ID NO:51, SEQ ID NO:52, and SEQ ID NO:53.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising amino acid sequences SEQ ID NO:54, SEQ ID NO:55, and SEQ ID NO:56.

10 In some embodiments, the anti-C1s antibody comprises a CDR-L1 having amino acid sequence SEQ ID NO:51, a CDR-L2 having amino acid sequence SEQ ID NO:52, a CDR-L3 having amino acid sequence SEQ ID NO:53, a CDR-H1 having amino acid sequence SEQ ID NO:54, a CDR-H2 having amino acid sequence SEQ ID NO:55, and a CDR-H3 having amino acid sequence SEQ ID NO:56.

15 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in SEQ ID NO:57.

SEQ ID NO. 57:

20 DIVMTQTAIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLAS  
GVPARFSGSGSGTFYSLTISSMEAEDDATYYCHQYYRLPPITFGAGTKLELK.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in SEQ ID NO. 58.

25 SEQ ID NO. 58:

QVKLEESGGALVKPGGSLKLSCAASGFTFSNYAMSWVRQIPEKRLEWVATISSGGSH  
TYYLDSVKGRFTISRDNARDTLYLQMSSLRSEDTALYYCARLFTGYAMDYWGQGTS  
VT.

30 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:57.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:58.

5 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising amino acid sequence SEQ ID NO:57.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising amino acid sequence SEQ ID NO:58.

10 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:57 and a heavy chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:58.

In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising amino acid sequence SEQ ID NO:57 and a heavy chain variable region comprising amino acid sequence SEQ ID NO:58.

15 In some embodiments, the anti-C1s antibody specifically binds an epitope within the complement C1s protein, wherein the antibody competes for binding the epitope with an antibody that comprises light chain CDRs of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:57 and heavy chain CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:58.

20 In some embodiments, the anti-C1s antibody comprises light chain CDRs of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:57 and heavy chain CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:58.

25 In some embodiments, the anti-C1s antibody (*e.g.*, a subject antibody that specifically binds an epitope in a complement C1s protein) comprises: a) a light chain region comprising one, two, or three CDRs selected from SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:53; and b) a heavy chain region comprising one, two, or three CDRs selected from SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66.

SEQ ID NO.62: TASSSVSSSYLH;

30 SEQ ID NO. 63: STSNLAS;

SEQ ID NO.53: HQYYRLPPIT;

SEQ ID NO.64: NYAMS;

SEQ ID NO.65: TISSGGSHTYYLDSVKG;

SEQ ID NO.66: LFTGYAMDY

In some embodiments, the anti-C1s antibody comprises a CDR having an amino acid  
5 sequence selected from SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:53, SEQ ID NO:64,  
SEQ ID NO:65, and SEQ ID NO:66.

In some embodiments, the anti-C1s antibody comprises a light chain variable region  
comprising amino acid sequences SEQ ID NO:62, SEQ ID NO:63, and SEQ ID NO:53.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region  
10 comprising amino acid sequences SEQ ID NO:64, SEQ ID NO:65, and SEQ ID NO:66.

In some embodiments, the anti-C1s antibody comprises a CDR-L1 having amino acid  
sequence SEQ ID NO:62, a CDR-L2 having amino acid sequence SEQ ID NO:63, a CDR-L3  
having amino acid sequence SEQ ID NO:53, a CDR-H1 having amino acid sequence SEQ ID  
NO:64, a CDR-H2 having amino acid sequence SEQ ID NO:65, and a CDR-H3 having  
15 amino acid sequence SEQ ID NO:66.

In some embodiments, the anti-C1s antibody comprises a light chain variable region  
comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,  
93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in  
SEQ ID NO:67.

20 SEQ ID NO. 67:

QIVLTQSPAIMSASLGERVTMTCTASSSVSSSYLHWYQQKPGSSPKLWIYSTSNLASG  
VPA RFSGSGSGTFYSLTISSMEAEDDATYYCHQYYRLPPITFGAGTKLELK.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region  
comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,  
25 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in  
SEQ ID NO:68.

SEQ ID NO. 68:

EVMLVESGGALVKPGGSLKLSCAASGFTFSNYAMSWVRQIPEKRLEWVATISSGGSH  
30 TYYLDSVKGRFTISRDNARDTLYLQMSSLRSEDALYYCARLFTGYAMDYWGQGTS  
VTVSS.

In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:67.

5 In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:68.

In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising amino acid sequence SEQ ID NO:67.

10 In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising amino acid sequence SEQ ID NO:68.

In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:67 and a heavy chain variable region comprising an amino acid sequence that is 90% identical to amino acid sequence SEQ ID NO:68.

15 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 95% identical to amino acid sequence SEQ ID NO:67 and a heavy chain variable region comprising an amino acid sequence that is 95% identical to amino acid sequence SEQ ID NO:68.

20 In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising amino acid sequence SEQ ID NO:67 and a heavy chain variable region comprising amino acid sequence SEQ ID NO:68.

25 In some embodiments, the anti-C1s antibody specifically binds an epitope within the complement C1s protein, wherein the antibody competes for binding the epitope with an antibody that comprises light chain CDRs of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:67 and heavy chain CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:68.

30 In some embodiments, the anti-C1s antibody comprises light chain CDRs of an antibody light chain variable region comprising amino acid sequence SEQ ID NO:67 and heavy chain CDRs of an antibody heavy chain variable region comprising amino acid sequence SEQ ID NO:68.

In some embodiments, the anti-C1s antibody comprises a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in SEQ ID NO:67.

In some embodiments, the anti-C1s antibody comprises a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%,  
5 93%, 94%, 95%, 96%, 97%, 98% or 99% identical to the amino acid sequence set forth in SEQ ID NO:68.

An anti-C1s antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,  
10 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:79 and depicted in FIG. 2 (VH variant 1).

An anti-C1s antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,  
15 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:80 and depicted in FIG. 3 (VH variant 2).

An anti-C1s antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,  
20 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:81 and depicted in FIG. 4 (VH variant 3).

An anti-C1s antibody can comprise a heavy chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%,  
25 96%, 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:82 and depicted in FIG. 5 (VH variant 4).

An anti-C1s antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,  
30 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:83 and depicted in FIG. 6 (VK variant 1).

An anti-C1s antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,  
35 97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:84 and depicted in FIG. 7 (VK variant 2).

An anti-C1s antibody can comprise a light chain variable region comprising an amino acid sequence that is 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%,

97%, 98%, 99%, or 100% identical to the amino acid sequence set forth in SEQ ID NO:85 and depicted in FIG. 8 (VK variant 3).

An anti-C1s antibody can comprise a heavy chain variable region comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 of the framework (FR) amino acid substitutions, relative to the  
5 IPN003 parental antibody FR amino acid sequences, depicted in Table 3 (FIG. 9).

### Definitions

As used herein the specification, “a” or “an” may mean one or more. As used herein in the claim(s), when used in conjunction with the word “comprising”, the words “a” or “an”  
10 may mean one or more than one. For example, reference to an “antibody” is a reference from one to many antibodies. As used herein “another” may mean at least a second or more.

As used herein, administration “*conjointly*” 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  
15 administration as separate compositions, including at different dosing frequencies or intervals, and using the same route of administration or different routes of administration.

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  
20 from at least two intact antibodies, and antibody fragments so long as they exhibit the desired biological activity.

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  
25 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.

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  
30 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 (“ $\alpha$ ”), delta (“ $\delta$ ”), epsilon (“ $\epsilon$ ”), gamma (“ $\gamma$ ”) and mu (“ $\mu$ ”), respectively. The  $\gamma$  and  $\alpha$  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  
5 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).

“*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  
10 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 ( $V_H$ ) followed by a number of constant domains. Each light chain has a variable domain at one end ( $V_L$ ) and a constant domain at its other end;  
15 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.

An “*isolated*” molecule or cell is a molecule or a cell that is identified and separated  
20 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;  
25 isolated cells are distinguished from cells existing naturally in tissues, organs, or individuals. In some embodiments, the isolated molecule is an anti-C1s, anti-C1q, or anti-C1r antibody of the present disclosure. In other embodiments, the isolated cell is a host cell or hybridoma cell producing an anti-C1s, anti-C1q, or anti-C1r antibody of the present disclosure.

An “*isolated*” antibody is one that has been identified, separated and/or recovered  
30 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 certain 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  
5   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. An isolated antibody includes the antibody *in situ* within recombinant T-cells since at least one component of the antibody's natural  
10   environment will not be present. Ordinarily, however, an isolated polypeptide or antibody will be prepared by a process including at least one purification step.

The “*variable region*” or “*variable domain*” of an antibody 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  
15   domains are generally the most variable parts of the antibody (relative to other antibodies of the same class) and contain the antigen binding sites.

The term “*variable*” refers to the fact that certain segments of the variable domains differ extensively in sequence among antibodies. The V domain mediates antigen binding and defines the specificity of a particular antibody for its particular antigen. However, the  
20   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,  
25   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  
30   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.

As used herein, the term “CDR” or “complementarity determining region” is intended to mean the non-contiguous antigen binding sites found within the variable region of both

heavy and light chain polypeptides. CDRs have been described by Kabat et al., J. Biol. Chem. 252:6609-6616 (1977); Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of proteins of immunological interest" (1991) (also referred to herein as Kabat 1991); by Chothia et al., J. Mol. Biol. 196:901-917 (1987) (also referred to herein as Chothia 1987);  
 5 and MacCallum et al., J. Mol. Biol. 262:732-745 (1996), where the definitions include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or grafted antibodies or variants thereof is intended to be within the scope of the term as defined and used herein. The amino acid residues, which encompass the CDRs, as defined by each of the  
 10 above cited references are set forth below in Table 1 as a comparison. The CDRs listed in Table 2 were defined in accordance with Kabat 1991.

**Table 1**

## CDR Definitions

|                      | Kabat <sup>1</sup> | Chothia <sup>2</sup> | MacCallum <sup>3</sup> |
|----------------------|--------------------|----------------------|------------------------|
| V <sub>H</sub> CDR-1 | 31-35              | 26-32                | 30-35                  |
| V <sub>H</sub> CDR-2 | 50-65              | 53-55                | 47-58                  |
| V <sub>H</sub> CDR-3 | 95-102             | 96-101               | 93-101                 |
| V <sub>L</sub> CDR-1 | 24-34              | 26-32                | 30-36                  |
| V <sub>L</sub> CDR-2 | 50-56              | 50-52                | 46-55                  |
| V <sub>L</sub> CDR-3 | 89-97              | 91-96                | 89-96                  |

<sup>1</sup>Residue numbering follows the nomenclature of Kabat et al., supra

<sup>2</sup>Residue numbering follows the nomenclature of Chothia et al., supra

<sup>3</sup>Residue numbering follows the nomenclature of MacCallum et al., supra

Table 2

| Antibody   | CDR-1                        | CDR-2                      | CDR-3                       | V region                                                                                                                                        |
|------------|------------------------------|----------------------------|-----------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------|
| IPN-M34 VL | SEQ ID NO.51<br>SSVSSSYLHWYQ | SEQ ID NO.52<br>STSNLASGVP | SEQ ID NO.53<br>HQYYRLPPIT  | SEQ ID NO.57<br>DIVMTQTAIMSASLGERVTMTCTASSSVSSSYL<br>HWYQQKPGSSPKLWIYSTSNLASGVPARFSGSG<br>SGTFYSLTISSEAEEDDATYYCHQYYRLPPITFG<br>AGTKLELK        |
| IPN-M34 VH | SEQ ID NO.54<br>GFTFSNYAMSWV | SEQ ID NO.55<br>ISSGGSHTYY | SEQ ID NO.56<br>ARLFTGYAMDY | SEQ ID NO.58<br>QVKLEESGGALVMPGGSLKLSCAASGFTFSNYA<br>MSWVRQIPEKRLEWVATISSGGSHTYYLDSVK<br>GRFTISRDNARDTLYLQMSSLRSEDTALYYCAR<br>LFTGYAMDYWGQGTSVT |

As used herein, the terms “CDR-L1”, “CDR-L2”, and “CDR-L3” refer, respectively, to the first, second, and third CDRs in a light chain variable region. As used herein, the terms “CDR-H1”, “CDR-H2”, and “CDR-H3” refer, respectively, to the first, second, and third CDRs in a heavy chain variable region. As used herein, the terms “CDR-1”, “CDR-2”, and “CDR-3” refer, respectively, to the first, second and third CDRs of either chain's variable region.

The term “*monoclonal antibody*” as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies of 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, monoclonal antibodies are advantageous since they are typically synthesized by hybridoma culture, uncontaminated by other immunoglobulins. The modifier “monoclonal” indicates the character of the antibody as being obtained as 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 disclosure 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).

The terms “*full-length antibody*,” “*intact antibody*” and “*whole antibody*” are used interchangeably to refer to an antibody 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.

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.

Papain digestion of antibodies 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>H</sub>1 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 with  
5 hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

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  
10 types of cells.

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  
15 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,  
20 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 disclosure include human IgG1, IgG2, IgG3 and IgG4.

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  
25 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.

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  
30 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.

5       “*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  
10   “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  
15   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.

Binding to FcRn *in vivo* and serum half-life of human FcRn high-affinity binding  
20   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).

25       “*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  
30   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.

“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. 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).

“Functional fragments” of antibodies 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.

The term “diabodies” refers to small antibody fragments prepared by constructing sFv 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 described in greater detail in, for example, EP 404,097; WO 1993/011161; WO/2009/121948; WO/2014/191493; Hollinger et al., *Proc. Nat’l Acad. Sci. USA* 90:6444-48 (1993).

As used herein, a “chimeric antibody” refers to an antibody (immunoglobulin) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is(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 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.”

“Humanized” forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies that contain minimal sequence derived from non-human immunoglobulin. In some

embodiments, 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  
5 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  
10 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  
15 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  
20 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.

A “*human antibody*” is one that possesses an amino-acid sequence corresponding to that of an antibody produced by a human and/or has been made using any of the techniques  
25 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  
30 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.

The term “hypervariable region,” “HVR,” or “HV,” when used herein refers to the regions of an antibody-variable domain 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).

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                    |

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.

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

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 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.

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, 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, 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).

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 fewer, 9 or fewer, 8 or fewer, 7 or fewer, 6 or fewer, 5 or fewer, 4 or fewer, 3 or fewer, or 2 or fewer. 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 some embodiments, the VL acceptor human framework is identical in sequence to the VL human immunoglobulin framework sequence or human consensus framework sequence.

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*.

An “*amino-acid modification*” at a specified position 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.

An “*affinity-matured*” antibody 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 some embodiments, 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).

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 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 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 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.

“*Identity*”, as used herein, indicates that at any particular position in the aligned sequences, the amino acid residue is identical between the sequences. “*Similarity*”, as used herein, indicates that, at any particular position in the aligned sequences, the amino acid residue is of a similar type between the sequences. For example, leucine may be substituted for isoleucine or valine. Other amino acids which can often be substituted for one another include but are not limited to:

- phenylalanine, tyrosine and tryptophan (amino acids having aromatic side chains);
- lysine, arginine and histidine (amino acids having basic side chains);
- aspartate and glutamate (amino acids having acidic side chains);
- asparagine and glutamine (amino acids having amide side chains); and
- cysteine and methionine (amino acids having sulphur-containing side chains).

Degrees of identity and similarity can be readily calculated. (See e.g., Computational Molecular Biology, Lesk, A.M., ed., Oxford University Press, New York, 1988; Biocomputing. Informatics and Genome Projects, Smith, D.W., ed., Academic Press, New York, 1993; Computer Analysis of Sequence Data, Part 1, Griffin, A.M., and Griffin, H.G., eds., Humana Press, New Jersey, 1994; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991)

As used herein, an “*interaction*” between a complement protein 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.

A “*blocking*” antibody, an “*antagonist*” antibody, an “*inhibitory*” antibody, or a “*neutralizing*” antibody is an antibody 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.

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.

As used herein, the term “affinity” refers to the equilibrium constant for the reversible binding of two agents (e.g., an antibody and an antigen) and is expressed as a dissociation constant (KD). Affinity can be at least 1-fold greater, at least 2-fold greater, at least 3-fold greater, at least 4-fold greater, at least 5-fold greater, at least 6-fold greater, at least 7-fold greater, at least 8-fold greater, at least 9-fold greater, at least 10-fold greater, at least 20-fold greater, at least 30-fold greater, at least 40-fold greater, at least 50-fold greater, at least 60-fold greater, at least 70-fold greater, at least 80-fold greater, at least 90-fold greater, at least 100-fold greater, or at least 1,000-fold greater, or more, than the affinity of an antibody for unrelated amino acid sequences. Affinity of an antibody to a target protein can be, for

example, from about 100 nanomolar (nM) to about 0.1 nM, from about 100 nM to about 1 picomolar (pM), or from about 100 nM to about 1 femtomolar (fM) or more. As used herein, the term “avidity” refers to the resistance of a complex of two or more agents to dissociation after dilution. The terms “immunoreactive” and “preferentially binds” are used interchangeably herein with respect to antibodies and/or antigen-binding fragments.

The term “binding” refers to a direct association between two molecules, due to, for example, covalent, electrostatic, hydrophobic, and ionic and/or hydrogen-bond interactions, including interactions such as salt bridges and water bridges. For example, a subject anti-C1s antibody binds specifically to an epitope within a complement C1s protein. “Specific binding” refers to binding with an affinity of at least about  $10^{-7}$  M or greater, *e.g.*,  $5 \times 10^{-7}$  M,  $10^{-8}$  M,  $5 \times 10^{-8}$  M, and greater. “Non-specific binding” refers to binding with an affinity of less than about  $10^{-7}$  M, *e.g.*, binding with an affinity of  $10^{-6}$  M,  $10^{-5}$  M,  $10^{-4}$  M, etc.

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

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.

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

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.

A “biological sample” encompasses a variety of sample types obtained from an individual and can be used in a diagnostic or monitoring assay. The definition encompasses blood and other liquid samples of biological origin, solid tissue samples such as a biopsy

specimen or tissue cultures or cells derived therefrom and the progeny thereof. The definition also includes samples that have been manipulated in any way after their procurement, such as by treatment with reagents, solubilization, or enrichment for certain components, such as polynucleotides. The term “biological sample” encompasses a clinical sample, and also includes cells in culture, cell supernatants, cell lysates, serum, plasma, biological fluid, and tissue samples. The term “biological sample” includes urine, saliva, cerebrospinal fluid, interstitial fluid, ocular fluid, synovial fluid, blood fractions such as plasma and serum, and the like. The term “biological sample” also includes solid tissue samples, tissue culture samples, and cellular samples.

An “*isolated*” nucleic acid molecule 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 acids encoding any polypeptides and antibodies herein that exist naturally in cells.

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 (*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 useful in recombinant DNA techniques 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.

“*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 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) 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, phosphoamidates, carbamates, *etc.*) and with charged linkages (*e.g.*, phosphorothioates, phosphorodithioates, *etc.*), those containing pendant moieties, such as, for example, proteins (*e.g.*, nucleases, toxins, antibodies, signal peptides, poly-L-lysine, *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 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 aralkyl. Not all linkages in a polynucleotide need be identical. The preceding description applies to all polynucleotides referred to herein, including RNA and DNA.

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 disclosure.

“*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™, polyethylene glycol (PEG), and PLURONICS™.

The term “*neurotrophins*” refers to neurotrophic factors that are neuroprotective in the brain. These factors are suitable for use in the compositions and methods of the disclosure and include brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-4/5, fibroblast growth factor (FGF)-2 and other FGFs, neurotrophin (NT)-3, erythropoietin (EPO), hepatocyte growth factor (HGF), epidermal growth factor (EGF), transforming growth factor (TGF)- $\alpha$ , TGF- $\beta$ , vascular endothelial growth factor (VEGF), interleukin-1 receptor antagonist (IL-1ra), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurturin, platelet-derived growth factor (PDGF), heregulin, neuregulin, artemin, persephin, interleukins, granulocyte-colony stimulating factor (CSF), granulocyte-macrophage-CSF, netrins, cardiotrophin-1, hedgehogs, leukemia inhibitory

factor (LIF), midkine, pleiotrophin, bone morphogenetic proteins (BMPs), saposins, semaphorins, and stem cell factor (SCF).

The term “*preventing*” is art-recognized, and when used in relation to a condition, such as an FTD disease, is well understood in the art, and includes administration of a composition which reduces the frequency or severity, or delays the onset, of one or more symptoms of the medical condition in a subject relative to a subject who does not receive the composition. Thus, the prevention of FTD disease progression includes, for example, slowing the average amount of neurodegeneration in a population of patients receiving a therapy relative to a control population that did not receive the therapy, *e.g.*, by a statistically and/or clinically significant amount. Similarly, the prevention of neurodegenerative disease progression includes reducing the likelihood that a patient receiving a therapy will develop a disability, such as cognitive decline and/or memory loss, or delaying the onset of disability, relative to a patient who does not receive the therapy.

The term “*subject*” as used herein refers to a living mammal and may be interchangeably used with the term “*patient*”. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. The term does not denote a particular age or gender.

As used herein, the term “*treating*” or “*treatment*” includes reducing, arresting, or reversing the symptoms, clinical signs, or underlying pathology of a condition to stabilize or improve a subject's condition or to reduce the likelihood that the subject's condition will worsen as much as if the subject did not receive the treatment.

The term “*therapeutically effective amount*” of a compound with respect to the subject method of treatment refers to an amount of the compound(s) in a preparation which, when administered as part of a desired dosage regimen (to a mammal, preferably a human) alleviates a symptom, ameliorates a condition, or slows the onset of disease conditions according to clinically acceptable standards for the disorder or condition to be treated or the cosmetic purpose, *e.g.*, at a reasonable benefit/risk ratio applicable to any medical treatment. A therapeutically effective amount herein may vary according to factors such as the disease state, age, sex, and weight of the patient, and the ability of the antibody to elicit a desired response in the individual.

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 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.

“*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 administered consecutively without interruption, but rather is cyclic/periodic in nature.

As used herein, administration “*conjointly*” with another compound or composition includes simultaneous administration and/or administration at different times. Conjoint administration 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.

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the present invention, the preferred methods and materials are now described. All publications mentioned herein are incorporated herein by reference to disclose and describe the methods and/or materials in connection with which the publications are cited. 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);  
5 *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,  
10 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).

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

Antibodies suitable for use in the methods 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 antibodies of the present disclosure are provided. Such nucleic acids may  
20 encode an amino acid sequence containing the V<sub>L</sub>/C<sub>L</sub> and/or an amino acid sequence containing the V<sub>H</sub>/C<sub>H1</sub> of the anti-C1q, anti-C1r or anti-C1s antibody. In some embodiments, one or more vectors (*e.g.*, expression vectors) containing such nucleic acids are provided. A host cell containing such nucleic acid may also be provided. The host cell may contain (*e.g.*, has been transduced with): (1) a vector containing a nucleic acid that encodes an amino acid  
25 sequence containing the V<sub>L</sub>/C<sub>L</sub> of the antibody and an amino acid sequence containing the V<sub>H</sub>/C<sub>H1</sub> of the antibody, or (2) a first vector containing a nucleic acid that encodes an amino acid sequence containing the V<sub>L</sub>/C<sub>L</sub> of the antibody and a second vector containing a nucleic acid that encodes an amino acid sequence containing the V<sub>H</sub>/C<sub>H1</sub> of the antibody. In some embodiments, the host cell is eukaryotic, *e.g.*, a Chinese Hamster Ovary (CHO) cell or  
30 lymphoid cell (*e.g.*, Y0, NS0, Sp20 cell).

Methods of making an anti-C1q, anti-C1r or anti-C1s antibody are disclosed herein. The method includes culturing a host cell of the present disclosure containing a nucleic acid

encoding the anti-C1q, anti-C1r or 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).

For recombinant production of a humanized anti-C1q, anti-C1r or anti-C1s antibody of the present disclosure, a nucleic acid encoding the 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).

Suitable vectors containing a nucleic acid sequence encoding any of the 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, Stratagene, and Invitrogen.

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-C1q, anti-C1r or anti-C1s antibody of the present disclosure.

Suitable host cells for cloning or expression of antibody-encoding vectors include prokaryotic or eukaryotic cells. For example, an anti-C1q, anti-C1r or anti-C1s antibody 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  
5 may be isolated from the bacterial cell paste in a soluble fraction and can be further purified.

### *Antibody Screening*

Candidate antibodies can be screened for the ability to modulate synapse loss. Such screening may be performed using an *in vitro* model, a genetically altered cell or animal, or  
10 purified protein. A wide variety of assays may be used for this purpose, such as an *in vitro* culture system. For example, an *in vitro* culture system may include the addition of microglial cells to cultures of cortical neurons, followed by counting the number of synapses removed from the neurons and/or ingested by the microglial cells.

Functional activity of a candidate antibody may also be tested *in vivo* by assessing the  
15 ability of the antibody to modulate synapse loss during normal aging, *e.g.*, in animals challenged with intracerebral injection of amyloid-beta oligomers, or in animals genetically modified with human familial mutations associated with FTD or in animals with induced forms of FTD.

Candidate antibodies may also be identified using computer-based modeling, by  
20 binding assays, and the like. Various *in vitro* models may be used to determine whether an antibody binds to, or otherwise affects complement activity. Such candidate antibodies may be tested by contacting neurons in an environment permissive for synapse loss. Such antibodies may be further tested in an *in vivo* model for an effect on synapse loss.

Synapse loss may be quantitated by administering the candidate antibodies to neurons  
25 in culture, and determining the presence of synapses in the absence or presence of the antibodies. In some embodiments of the disclosure, the neurons are a primary culture, *e.g.*, of retinal ganglion cells (RGCs). Purified populations of RGCs are obtained by conventional methods, such as sequential immunopanning. The cells are cultured in suitable medium, which will usually comprise appropriate growth factors, *e.g.*, CNTF; BDNF; *etc.* The neural  
30 cells, *e.g.*, RGCs, are cultured for a period of time sufficient allow robust process outgrowth and then cultured with a candidate antibodies for a period of about 1 day to 1 week. The neurons may be cultured on a live astrocyte cell feeder in order to induce signaling for synapse loss. Methods of culturing astrocyte feeder layers are known in the art. For example,

cortical glia can be plated in a medium that does not allow neurons to survive, with removal of non-adherent cells.

For synapse quantification, cultures are fixed, blocked and washed, then stained with an antibody specific for synaptic proteins, *e.g.*, synaptotagmin, etc. and visualized with an appropriate reagent. Analysis of the staining may be performed microscopically. In some embodiments, digital images of the fluorescence emission are with a camera and image capture software, adjusted to remove unused portions of the pixel value range and the used pixel values adjusted to utilize the entire pixel value range. Corresponding channel images may be merged to create a color (RGB) image containing the two single-channel images as individual color channels. Co-localized puncta can be identified using a rolling ball background subtraction algorithm to remove low-frequency background from each image channel. Number, mean area, mean minimum and maximum pixel intensities, and mean pixel intensities for all synaptotagmin, PSD-95, and colocalized puncta in the image are recorded for analysis.

Generally, a plurality of assay mixtures are run in parallel with different antibody concentrations to obtain a differential response to the various concentrations. Typically one of these concentrations serves as a negative control, *i.e.*, at zero concentration or below the level of detection.

#### *Pharmaceutical Compositions and Administration*

An antibody of the present disclosure may be administered in the form of pharmaceutical compositions.

Therapeutic formulations of an antibody of the disclosure may be prepared for storage by mixing the antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (*Remington's Pharmaceutical Sciences* 16th edition, Osol, A. Ed. [1980]), in the form of lyophilized formulations or aqueous solutions. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride, benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3-pentanol; and m-cresol); low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum

albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, histidine, arginine, or lysine; monosaccharides, disaccharides, and other carbohydrates including glucose, mannose, or dextrins; chelating agents such as EDTA; sugars such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions such as sodium; metal complexes (e.g., Zn-protein complexes); and/or non-ionic surfactants such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

Lipofections or liposomes may also be used to deliver an antibody or antibody fragment into a cell, wherein the epitope or smallest fragment which specifically binds to the binding domain of the target protein is preferred.

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

The formulations to be used for parenteral administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes.

Sustained-release preparations may be prepared. Suitable examples of sustained-release preparations include semipermeable matrices of solid hydrophobic polymers containing the antibody, which matrices are in the form of shaped articles, e.g., films, or microcapsules. Examples of sustained-release matrices include polyesters, hydrogels (for example, poly(2-hydroxyethyl-methacrylate), or poly(vinylalcohol)), polylactides (U.S. Pat. No. 3,773,919), copolymers of L-glutamic acid and  $\gamma$  ethyl-L-glutamate, non-degradable ethylene-vinyl acetate, degradable lactic acid-glycolic acid copolymers such as the LUPRON DEPOT™ (injectable microspheres composed of lactic acid-glycolic acid copolymer and leuprolide acetate), and poly-D-(-)-3-hydroxybutyric acid. While polymers such as ethylene-vinyl acetate and lactic acid-glycolic acid enable release of molecules for over 100 days, certain hydrogels release proteins for shorter time periods.

The antibodies and compositions of the present disclosure are typically administered by various routes, including, but not limited to, topical, parenteral, subcutaneous, intraperitoneal, intrapulmonary, intranasal, and intralesional administration. Parenteral routes

of administration include intramuscular, intravenous, intra-arterial, intraperitoneal, intrathecal, or subcutaneous administration.

Pharmaceutical compositions may also include, depending on the formulation desired, pharmaceutically-acceptable, non-toxic carriers or diluents, which are defined as vehicles  
5 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 are distilled water, buffered water, physiological saline, PBS, Ringer's solution, dextrose solution, and Hank's solution. In addition, the pharmaceutical composition or formulation may include other carriers, adjuvants, or non-  
10 toxic, nontherapeutic, non-immunogenic stabilizers, excipients and the like. The compositions may also include additional substances to approximate physiological conditions, such as pH adjusting and buffering agents, toxicity adjusting agents, wetting agents and detergents.

The composition may also include any of a variety of stabilizing agents, such as an  
15 antioxidant for example. When the pharmaceutical composition includes a polypeptide, the polypeptide may 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, enhance other pharmacokinetic and/or pharmacodynamic characteristics, or enhance solubility or uptake). Examples of such  
20 modifications or complexing agents include sulfate, gluconate, citrate and phosphate. The polypeptides of a composition may also be complexed with molecules that enhance their *in vivo* attributes. Such molecules include, for example, carbohydrates, polyamines, amino acids, other peptides, ions (*e.g.*, sodium, potassium, calcium, magnesium, manganese), and lipids. Further guidance regarding formulations that are suitable for various types of  
25 administration may 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).

Toxicity and therapeutic efficacy of the active ingredient may be determined according to standard pharmaceutical procedures in cell cultures and/or experimental  
30 animals, including, for example, determining the LD<sub>50</sub> (the dose lethal to 50% of the population) and the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it may be

expressed as the ratio LD50/ED50. Compounds that exhibit large therapeutic indices are preferred.

The data obtained from cell culture and/or animal studies may be used in formulating a range of dosages for humans. The dosage of the active ingredient typically lies within a range of circulating concentrations that include the ED50 with low toxicity. The dosage may vary within this range depending upon the dosage form employed and the route of administration utilized.

The pharmaceutical compositions described herein may be administered in a variety of different ways. Examples include administering a composition containing a pharmaceutically acceptable carrier via oral, intranasal, rectal, topical, intraperitoneal, intravenous, intramuscular, subcutaneous, subdermal, transdermal, intrathecal, and intracranial methods.

For oral administration, the active ingredient may 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) may 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 may be used to make compressed tablets. Both tablets and capsules may be manufactured as sustained release products to provide for continuous release of medication over a period of hours. Compressed tablets may 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 may contain coloring and flavoring to increase patient acceptance.

Formulations suitable for parenteral administration include aqueous and non-aqueous, isotonic sterile injection solutions, which may 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 may include suspending agents, solubilizers, thickening agents, stabilizers, and preservatives.

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 parenteral 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 typically substantially isotonic and made under GMP conditions.

The compositions of the disclosure may be administered using any medically appropriate procedure, *e.g.*, intravascular (intravenous, intraarterial, intracapillary) administration, injection into the cerebrospinal fluid, intravitreal, topical, intracavity or direct injection in the brain. Intrathecal administration may be carried out through the use of an Ommaya reservoir, in accordance with known techniques. (F. Balis et al., *Am J. Pediatr. Hematol. Oncol.* 11, 74, 76 (1989).

Where the therapeutic agents are locally administered in the brain, one method for administration of the therapeutic compositions of the disclosure is by deposition into or near the site by any suitable technique, such as by direct injection (aided by stereotaxic positioning of an injection syringe, if necessary) or by placing the tip of an Ommaya reservoir into a cavity, or cyst, for administration. Alternatively, a convection-enhanced delivery catheter may be implanted directly into the site, into a natural or surgically created cyst, or into the normal brain mass. Such convection-enhanced pharmaceutical composition delivery devices greatly improve the diffusion of the composition throughout the brain mass. The implanted catheters of these delivery devices utilize high-flow microinfusion (with flow rates in the range of about 0.5 to 15.0  $\mu\text{l}/\text{minute}$ ), rather than diffusive flow, to deliver the therapeutic composition to the brain and/or tumor mass. Such devices are described in U.S. Pat. No. 5,720,720, incorporated fully herein by reference.

The effective amount of a therapeutic composition given to a particular patient may depend on a variety of factors, several of which may be different from patient to patient. A competent clinician will be able to determine an effective amount of a therapeutic agent to administer to a patient. Dosage of the agent will depend on the treatment, route of administration, the nature of the therapeutics, sensitivity of the patient to the therapeutics, etc. Utilizing LD50 animal data, and other information, a clinician may determine the maximum

safe dose for an individual, depending on the route of administration. Utilizing ordinary skill, the competent clinician will be able to optimize the dosage of a particular therapeutic composition in the course of routine clinical trials. The compositions may be administered to the subject in a series of more than one administration. For therapeutic compositions, regular  
5 periodic administration will sometimes be required, or may be desirable. Therapeutic regimens will vary with the agent; for example, some agents may be taken for extended periods of time on a daily or semi-daily basis, while more selective agents may be administered for more defined time courses, *e.g.*, one, two three or more days, one or more weeks, one or more months, etc., taken daily, semi-daily, semi-weekly, weekly, etc.

10 Formulations may be optimized for retention and stabilization in the brain. 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  
15 increase in molecular weight.

Other strategies for increasing retention include the entrapment of the agent 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  
20 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  
25 or shape compatible with the selected site of insertion.

The implants may be monolithic, *i.e.*, having the active agent homogenously 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  
30 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.

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 may be condensation  
5 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  
10 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  
15 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 disclosure. Hydrogels are typically a copolymer material, characterized by the ability to imbibe a liquid.  
20 Exemplary biodegradable hydrogels which may be employed are described in Heller in: Hydrogels in Medicine and Pharmacy, N. A. Peppas ed., Vol. III, CRC Press, Boca Raton, Fla., 1987, pp 137-149.

### *Kits*

The present disclosure also provides a pharmaceutical pack or kit comprising one or  
25 more containers filled with one or more of the ingredients of the pharmaceutical compositions. Associated with such container(s) may be a notice in the form prescribed by a governmental agency regulating the manufacture, use or sale of pharmaceuticals or biological products, which notice reflects approval by the agency of manufacture, use or sale for human administration.

30 Kits of the present disclosure may include one or more containers comprising a purified anti-C1q, anti-C1r or 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 a disease, 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 FTD and the stage of the FTD.

5           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 present disclosure 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  
10       magnetic or optical storage disk) are also acceptable.

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

          The kits of this disclosure are preferably disposed in suitable packaging. Suitable packaging includes, but is not limited to, vials, bottles, jars, flexible packaging (*e.g.*, sealed  
15       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  
20       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.

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

### *Inhibition of complement*

          A number of molecules are known that inhibit the activity of complement. In addition  
30       to known compounds, suitable inhibitors can be screened by methods described herein. As described above, normal cells can produce proteins that block complement activity, *e.g.*,

CD59, C1 inhibitor, etc. In some embodiments of the disclosure, complement is inhibited by upregulating expression of genes encoding such polypeptides.

Modifications of molecules that block complement activation are also known in the art. For example, such molecules include, without limitation, modified complement receptors, such as soluble CR1. The mature protein of the most common allotype of CR1 contains 1998 amino acid residues: an extracellular domain of 1930 residues, a transmembrane region of 25 residues, and a cytoplasmic domain of 43 residues. The entire extracellular domain is composed of 30 repeating units referred to as short consensus repeats (SCRs) or complement control protein repeats (CCPRs), each consisting of 60 to 70 amino acid residues. Recent data indicate that C1q binds specifically to human CR1. Thus, CR1 recognizes all three complement opsonins, namely C3b, C4b, and C1q. A soluble version of recombinant human CR1 (sCR1) lacking the transmembrane and cytoplasmic domains has been produced and shown to retain all the known functions of the native CR1. The cardioprotective role of sCR1 in animal models of ischemia/reperfusion injury has been confirmed. Several types of human C1q receptors (C1qR) have been described. These include the ubiquitously distributed 60- to 67-kDa receptor, referred to as cC1qR because it binds the collagen-like domain of C1q. This C1qR variant was shown to be calreticulin; a 126-kDa receptor that modulates monocyte phagocytosis. gC1qR is not a membrane-bound molecule, but rather a secreted soluble protein with affinity for the globular regions of C1q, and may act as a fluid-phase regulator of complement activation.

Decay accelerating factor (DAF) (CD55) is composed of four SCRs plus a serine/threonine-enriched domain that is capable of extensive O-linked glycosylation. DAF is attached to cell membranes by a glycosyl phosphatidyl inositol (GPI) anchor and, through its ability to bind C4b and C3b, it acts by dissociating the C3 and C5 convertases. Soluble versions of DAF (sDAF) have been shown to inhibit complement activation.

C1 inhibitor, a member of the “serpin” family of serine protease inhibitors, is a heavily glycosylated plasma protein that prevents fluid-phase C1 activation. C1 inhibitor regulates the classical pathway of complement activation by blocking the active site of C1r and C1s and dissociating them from C1q.

Peptide inhibitors of complement activation include C5a and other inhibitory molecules include Fucan.

*Synapse loss*

Synapses are asymmetric communication junctions formed between two neurons, or, at the neuromuscular junction (NMJ) between a neuron and a muscle cell. Chemical synapses enable cell-to-cell communication via secretion of neurotransmitters, whereas in electrical synapses signals are transmitted through gap junctions, specialized intercellular channels that permit ionic current flow. In addition to ions, other molecules that modulate synaptic function (such as ATP and second messenger molecules) can diffuse through gap junctional pores. At the mature NMJ, pre- and postsynaptic membranes are separated by a synaptic cleft containing extracellular proteins that form the basal lamina. Synaptic vesicles are clustered at the presynaptic release site, transmitter receptors are clustered in junctional folds at the postsynaptic membrane, and glial processes surround the nerve terminal.

Synaptogenesis is a dynamic process. During development, more synapses are typically established than ultimately will be retained. Therefore, the elimination of excess synaptic inputs is a critical step in synaptic circuit maturation. Synapse elimination is a competitive process that involves interactions between pre- and postsynaptic partners. In the CNS, as with the NMJ, a developmental, activity-dependent remodeling of synaptic circuits takes place by a process that may involve the selective stabilization of coactive inputs and the elimination of inputs with uncorrelated activity. The anatomical refinement of synaptic circuits occurs at the level of individual axons and dendrites by a dynamic process that involves rapid elimination of synapses. As axons branch and remodel, synapses form and dismantle with synapse elimination occurring rapidly.

In addition to the normal developmental loss, synapse loss is an early pathological event common to many neurodegenerative disorders, including FTD, and is the best correlate to the cognitive impairment. For example, studies in the brains of patients with pre-clinical Alzheimer's disease (AD), as well as in transgenic animal models have shown that synaptic damage occurs early in disease progression. This early disruption of synaptic connections in the brain results in neuronal dysfunction that, in turn, leads to the characteristic symptoms of dementia and/or motor impairment observed in several neurodegenerative disorders.

Several molecules involved in AD and other neurodegenerative disorders play an important role in synaptic function. For example, A $\beta$ PP has a preferential localization at central and peripheral synaptic sites. In transgenic mice, abnormal expression of mutant forms of A $\beta$ PP results not only in amyloid deposition, but also in widespread synaptic damage. This synaptic pathology occurs early and is associated with levels of soluble A $\beta$ 1-42 rather than with plaque formation. Other neurodegenerative diseases where gene products

have been shown to be closely associated with synaptic complexes include Huntington's disease (HD) and myotonic dystrophy (DM). Huntingtin (HTT) is a membrane-bound protein with a distribution very similar to that of synaptic vesicle protein synaptophysin. Studies in human brain detected HTT in perikarya of some neurons, neuropil, varicosities and as punctate staining likely to be nerve endings. The serine/ threonine kinase (DMK), which is the gene product of the DM gene, has been found to localize post-synaptically at the neuromuscular junction of skeletal muscle and at intercalated discs of cardiac tissue. DMK was also found at synaptic sites in the cerebellum, hippocampus, midbrain and medulla.

Antibodies disclosed herein may be used to inhibit synapse loss. Inhibiting synapse loss results in maintenance of or reduced loss of synapses, where a decrease would otherwise occur.

#### *Blood Brain Barrier*

As used herein, the “*blood-brain barrier*” (BBB) refers to the barrier between the peripheral circulation and the brain and spinal cord. The BBB is formed by tight junctions within the brain capillary endothelial plasma membrane. The formation of such tight junctions creates an extremely tight barrier that restricts the transport of molecules into the brain, even molecules as small as urea, molecular weight of 60 Da. The blood-brain barrier within the brain, the blood-spinal cord barrier within the spinal cord, and the blood-retinal barrier within the retina, are contiguous capillary barriers within the central nervous system (CNS), and are collectively referred to as the blood-brain barrier or BBB. The disclosure provides compositions and methods that include an antibody that binds to a BBB receptor mediated transport system, coupled to an agent for which transport across the BBB is desired, *e.g.*, a neurotherapeutic agent. The compositions and methods of the disclosure may utilize any suitable structure that is capable of transport by the selected endogenous BBB receptor-mediated transport system, and that is also capable of attachment to the desired agent.

The BBB has been shown to have specific receptors that allow the transport from the blood to the brain of several macromolecules; these transporters are suitable as transporters for compositions of the disclosure. Endogenous BBB receptor-mediated transport systems useful in the disclosure include those that transport insulin, transferrin, insulin-like growth factors 1 and 2 (IGF1 and IGF2), leptin, and lipoproteins. In some embodiments, the disclosure utilizes a structure that is capable of crossing the BBB via the endogenous insulin

BBB receptor-mediated transport system, *e.g.*, the human endogenous insulin BBB receptor-mediated transport system.

One strategy for drug delivery through the blood brain barrier (BBB) entails disruption of the BBB, either by osmotic means such as mannitol or leukotrienes, or  
 5 biochemically by the use of vasoactive substances such as bradykinin. The potential for using BBB opening to target specific agents is also an option. A BBB disrupting agent can be co-administered with the therapeutic compositions of the disclosure when the compositions are administered by intravascular injection. Other strategies to go through the BBB may entail the use of endogenous transport systems, including carrier-mediated transporters such as  
 10 glucose and amino acid carriers, receptor-mediated transcytosis for insulin or transferrin, and active efflux transporters such as p-glycoprotein. Active transport moieties may also be conjugated to the antibodies of the disclosure to facilitate transport across the epithelial wall of the blood vessel. Alternatively, drug delivery behind the BBB may be pursued, *e.g.*, by intrathecal delivery of agents directly to the cerebrospinal fluid, as through an Ommaya  
 15 reservoir.

### *Frontotemporal Dementia*

The antibodies of the present disclosure may be useful in the present methods of preventing, reducing risk of developing, or treating FTD or a variant of FTD, comprising administering an inhibitor of the complement pathway (*e.g.*, an inhibitor, such as an anti-C1q,  
 20 -C1r, or -C1s antibody). The antibodies of the present disclosure may also be useful in inhibiting synapse loss in FTD.

Synapse loss is a significant correlate of cognitive decline that serves as a critical hallmark of neurodegenerative diseases. For example, microglia prune developing synapses and regulate synaptic plasticity and function. Disruptions in microglia–synapse interactions  
 25 contribute to synapse loss and dysfunction, including cognitive impairment in neurodegenerative diseases. Furthermore, disruption of immune-related molecules or receptors expressed on microglia, such as complement proteins or complement and fractalkine receptors, results in synaptic and wiring abnormalities in both prenatal and postnatal brain development implicating microglia in sculpting synaptic connectivity.

30 The etiology of frontotemporal dementia remains enigmatic. Frontotemporal lobar degeneration (FTLD) may be used as a pathological term used for the description of a clinically, pathologically and genetically heterogeneous group of disorders in which selective

degeneration of the frontal and temporal lobes is a prominent feature. The clinical term frontotemporal dementia (FTD) is used for the description of early onset dementias. FTD may overlap with motor neuron disease/amyotrophic lateral sclerosis (MND/ALS) (FTD-MND), Corticobasal syndrome (CBS) and progressive palsy (PSP) syndrome. FTD is also a highly heritable disorder with approximately 30-50% of cases reporting positive family history, although an autosomal dominant history accounts for approximately 10% of the cases. Mutations in three genes, microtubule-associated protein tau (MAPT), progranulin (GRN) and chromosome 9 open reading frame 72 (C9orf72) genes are considered responsible for most of the familial cases, and about 10–20% of all cases with FTD.

Frontotemporal dementia may be classified into three clinical variants: behavioral-variant frontotemporal dementia, which is associated with early behavioral and executive deficits; non-fluent variant primary progressive aphasia, with progressive deficits in speech, grammar, and word output; and semantic-variant primary progressive aphasia, which is a progressive disorder of semantic knowledge and naming. Clinically FTD patients can present with one of three canonical clinical syndromes: behavioral variant FTD (bvFTD), and two language variants, semantic dementia and progression non-fluent aphasia (PNFA). bvFTD is associated with early behavioral and executive deficits, which refers to the higher-level cognitive skills that control and coordinate other cognitive abilities and behaviors. Semantic dementia, also called semantic-variant primary progressive aphasia, is a progressive disorder of semantic knowledge and naming. PNFA is typically characterized by progressive deficits in speech, grammar, and word aphasia. As FTD progresses, the symptoms of the three clinical variants may converge, such as when an initially focal degeneration becomes more diffuse and spreads to affect large regions in the frontal and temporal lobes. Over time, patients develop global cognitive impairment and motor deficits, including Parkinsonism, and motor neuron disease in some patients. Patients with end-stage disease generally have difficulty eating, moving, and swallowing. Death usually happens about 8 years after symptom onset and typically caused by pneumonia or other secondary infections.

The most common pronounced early symptoms of behavioral variant frontotemporal dementia include personality changes, disinhibition, and apathy. Some individuals who meet diagnostic criteria for behavioral-variant frontotemporal dementia may have a very slow disease course (over decades) with slow progression of cognitive impairment and often normal MRI and PET studies. Their disease is classified as frontotemporal dementia phenocopy. Some of these individuals have a primary psychiatric disturbance such as bipolar

disorder, Asperger's syndrome, or factitious disease, whereas others might have a slow sporadic or genetic form of frontotemporal dementia

Patients with primary progressive aphasia typically have a progressive, insidious decline in linguistic skills during the initial phase of the disease. Language dysfunction is commonly one of the main symptoms for the first 2 years of the illness. Deficits include language production, object naming, syntax, or word comprehension, and are apparent during conversation or through speech and language assessment. Language deficit is commonly one of the main causes of impaired activities of daily living. Although the underlying cause is more often frontotemporal dementia, primary progressive aphasia can be associated with Alzheimer's disease.

Symptoms of semantic-variant primary progressive aphasia may result from early asymmetrical degeneration of anterior temporal lobes and amygdala. Semantic loss causes anomia for people, places, and objects; word-finding difficulties; and impaired word comprehension. Left temporal lobe variant presents with mainly linguistic semantic loss (semantic-variant primary).

About 12.5% of patients with behavioral-variant frontotemporal dementia develop motor neuron disease, typically including upper motor neuron signs (hyperreflexia, extensor plantar response, spasticity), lower motor neuron signs (weakness, muscle atrophy, fasciculations), dysarthria, dysphagia, and pseudobulbar affect. Mild features of motor neuron disease can occur in up to 40% of patients with frontotemporal dementia. Among the frontotemporal dementia variants, motor neuron disease arises frequently in patients with behavioral-variant frontotemporal dementia and less often in patients with semantic-variant primary progressive aphasia or nonfluent variant primary progressive aphasia. Early parkinsonism is present in up to 20% of patients with frontotemporal dementia and is most often seen in patients with behavioral-variant frontotemporal dementia, followed by those with non-fluent variant primary progressive aphasia. Patients with frontotemporal dementia have features of corticobasal syndrome or progressive supranuclear palsy syndrome. Corticobasal syndrome is characterized by asymmetrical parkinsonism, sensory-motor cortical dysfunction, alien-limb syndrome, and dystonia. Progressive supranuclear palsy syndrome is characterized by vertical supranuclear palsy, decreased saccade velocity, and early postural instability with falls. Behavioral changes, including executive dysfunction, apathy, and impulsivity, are common.

### *Methods of Treatment*

By administering agents that inhibit complement activation, synapses can be maintained that would otherwise be lost. Such agents include an anti-C1q, anti-C1r, or anti-C1s antibody inhibitor. Other agents may include inhibitors that upregulate expression of native complement, or agents that down-regulate C1q, C1r or C1s synthesis in neurons, astrocytes, microglia, endothelial, or oligodendroglial cells, agents that block complement activation, agents that block the signal for complement activation, and the like.

The methods promote improved maintenance of neuronal function in conditions associated with synapse loss. The maintenance of neural connections provides for functional improvement in neurodegenerative disease relative to untreated patients. The prevention of synapse loss may comprise at least a measurable improvement relative to a control lacking such treatment over the period of 1, 2, 3, 4, 5, 6 days or at least one week, for example at least a 10% improvement in the number of synapses, at least a 20% improvement, at least a 50% improvement, or more.

The agents of the present disclosure may be administered at a dosage that decreases synapse loss while minimizing any side-effects. It is contemplated that compositions may be obtained and used under the guidance of a physician for *in vivo* use. The dosage of the therapeutic formulation may vary widely, depending upon the nature of the disease, the frequency of administration, the manner of administration, the clearance of the agent from the host, and the like.

The effective amount of a therapeutic composition given to a particular patient may depend on a variety of factors, several of which may be different from patient to patient. Utilizing ordinary skill, the competent clinician will be able to tailor the dosage of a particular therapeutic or imaging composition in the course of routine clinical trials.

Therapeutic agents, *e.g.*, inhibitors of complement, activators of gene expression, etc. can be incorporated into a variety of formulations for therapeutic administration by combination with appropriate pharmaceutically acceptable carriers or diluents, and may be formulated into preparations in solid, semi-solid, liquid or gaseous forms, such as tablets, capsules, powders, granules, ointments, solutions, suppositories, injections, inhalants, gels, microspheres, and aerosols. As such, administration of the compounds can be achieved in various ways, including oral, buccal, rectal, parenteral, intraperitoneal, intradermal, transdermal, intrathecal, nasal, intratracheal, etc., administration. The active agent may be

systemic after administration or may be localized by the use of regional administration, intramural administration, or use of an implant that acts to retain the active dose at the site of implantation.

### *Combination Treatments*

5           The complement inhibitors of the present disclosure may be used, without limitation, conjointly with any additional treatment, such as immunosuppressive therapies, for treating FTD.

10           In some embodiments, the antibodies of this disclosure may be administered in 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 Compstatin-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 as nafamostat mesilate (FUTHAN; FUT-175), aprotinin, K-76 monocarboxylic acid  
15 (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  
20 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, C1r, or C1s binding sites in their Fc domains (*e.g.,* Fab fragments or antibodies otherwise engineered not to bind C1q, C1r, or C1s).

25           The methods of the present disclosure can find use in combination with cell or tissue transplantation to the central nervous system, where such grafts include neural progenitors such as those found in fetal tissues, neural stem cells, embryonic stem cells or other cells and tissues contemplated for neural repair or augmentation. Neural stem and progenitor cells can participate in aspects of normal development, including migration along well-established  
30 migratory pathways to disseminated CNS regions, differentiation into multiple developmentally- and regionally-appropriate cell types in response to microenvironmental cues, and non-disruptive, non-tumorigenic interspersions with host progenitors and their

progeny. Human NSCs are capable of expressing foreign transgenes *in vivo* in these disseminated locations. Accordingly, these cells find use in the treatment of FTD.

#### INCORPORATION BY REFERENCE

5           Each of the patents, published patent applications, and non-patent references cited herein are hereby incorporated by reference in their entirety.

#### EQUIVALENTS

10           Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the following claims.

15

What is claimed is:

1. A method of preventing, reducing risk of developing, or treating frontotemporal dementia (FTD) or a variant of FTD, comprising administering to a subject an inhibitor of the complement pathway.
- 5 2. The method of claim 1, wherein the variant of FTD is a behavioral variant, a semantic variant, a non-fluent variant, and/or primary progressive aphasia.
3. The method of claim 1, wherein the inhibitor is administered to a subject with social symptoms, emotional symptoms, eating and oral symptoms, repetitive or compulsive symptoms, sensory symptoms, motor symptoms, executive symptoms, language symptoms,  
10 and/or neuropsychiatric symptoms.
4. The method of any one of claims 1-3, wherein the inhibitor is an antibody.
5. The method of claim 4, wherein the antibody is an anti-C1q antibody.
6. The method of claim 5, wherein the anti-C1q antibody inhibits the interaction between C1q and an autoantibody or between C1q and C1r, or between C1q and C1s.
- 15 7. The method of claim 4, wherein the anti-C1q antibody promotes clearance of C1q from circulation or a tissue.
8. The method of any one of claims 5-7, wherein the antibody is an anti-C1q antibody having a dissociation constant ( $K_D$ ) that ranges from 100 nM to 0.005 nM or less than 0.005 nM.
- 20 9. The method of any one of claims 5-8, wherein the antibody is an anti-C1q antibody that binds C1q with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than 1.0:1.
10. The method of claim 9, wherein the antibody is an anti-C1q antibody that binds C1q with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than 1.0:1.
11. The method of claim 10, wherein the antibody is an anti-C1q antibody that binds C1q  
25 with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than 1.0:1.
12. The method of claim 4, wherein the antibody is an anti-C1r antibody.

13. The method of claim 12, wherein the anti-C1r antibody inhibits the interaction between C1r and C1q or between C1r and C1s, or wherein the anti-C1r antibody inhibits the catalytic activity of C1r or inhibits the processing of pro-C1r to an active protease.
14. The method of claim 12 or 13, wherein the antibody is an anti-C1r antibody having a  
5 dissociation constant ( $K_D$ ) that ranges from 100 nM to 0.005 nM or less than 0.005 nM.
15. The method of any one of claims 12-14, wherein the antibody is an anti-C1r antibody that binds C1r with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than 1.0:1.
16. The method of claim 15, wherein the antibody is an anti-C1r antibody that binds C1r with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than 1.0:1.
- 10 17. The method of claim 16, wherein the antibody is an anti-C1r antibody that binds C1r with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than 1.0:1.
18. The method of any one of claims 12 to 17, wherein the anti-C1r antibody promotes clearance of C1r from circulation or a tissue.
19. The method of claim 4, wherein the antibody is an anti-C1s antibody.
- 15 20. The method of claim of 19, wherein the anti-C1s antibody inhibits the interaction between C1s and C1q or between C1s and C1r or between C1s and C2 or C4, or wherein the anti-C1s antibody inhibits the catalytic activity of C1s or inhibits the processing of pro-C1s to an active protease.
21. The method of claim 19 or 20, wherein the antibody is an anti-C1s antibody having a  
20 dissociation constant ( $K_D$ ) that ranges from 100 nM to 0.005 nM or less than 0.005 nM.
22. The method of any one of claims 19-21, wherein the antibody is an anti-C1s antibody that binds C1s with a binding stoichiometry that ranges from 20:1 to 1.0:1 or less than 1.0:1.
23. The method of claim 22, wherein the antibody is an anti-C1s antibody that binds C1s with a binding stoichiometry that ranges from 6:1 to 1.0:1 or less than 1.0:1.
- 25 24. The method of claim 23, wherein the antibody is an anti-C1s antibody that binds C1s with a binding stoichiometry that ranges from 2.5:1 to 1.0:1 or less than 1.0:1.

25. The method of any one of claims 19 to 24, wherein the anti-C1s antibody promotes clearance of C1s from circulation or a tissue.

26. The antibody of any one of claims 4-24, wherein the antibody specifically binds to and neutralizes a biological activity of C1q.

5 27. The antibody of claim 26, wherein the biological activity is (1) C1q binding to an autoantibody, (2) C1q binding to C1r, (3) C1q binding to C1s, (4) C1q binding to phosphatidylserine, (5) C1q binding to pentraxin-3, (6) C1q binding to C-reactive protein (CRP), (7) C1q binding to globular C1q receptor (gC1qR), (8) C1q binding to complement receptor 1 (CR1), (9) C1q binding to beta-amyloid, (10) C1q binding to calreticulin, (11) C1q  
10 binding to apoptotic cells, or (12) C1q binding to components of a nerve cell membrane.

28. The antibody of claim 26 or 27, wherein the biological activity is (1) activation of the classical complement activation pathway, (2) activation of antibody and complement dependent cytotoxicity, (3) CH50 hemolysis, (4) synapse loss, (5) B-cell antibody production, (6) dendritic cell maturation, (7) T-cell proliferation, (8) cytokine production (9)  
15 microglia activation, (10) Arthus reaction, (11) phagocytosis of synapses or nerve endings, or (12) activation of complement receptor 3 (CR3/C3) expressing cells.

29. The method of claim 28, wherein CH50 hemolysis comprises human, mouse, rat, dog, rhesus, and/or cynomolgus monkey CH50 hemolysis.

30. The method of claim 28 or claim 29, wherein the antibody is capable of neutralizing  
20 from at least about 50%, to at least about 90% of CH50 hemolysis.

31. The method of any one of claims 28-30, wherein the antibody is capable of neutralizing at least 50% of CH50 hemolysis at a dose of less than 150 ng, less than 100 ng, less than 50 ng, or less than 20 ng.

32. The method of any preceding claim, wherein the antibody is a monoclonal antibody, a  
25 polyclonal antibody, a recombinant antibody, a humanized antibody, a chimeric antibody, a multispecific antibody, or an antibody fragment thereof.

33. The method of claim 32, wherein the antibody is an antibody fragment and the antibody fragment is a Fab fragment, a Fab' fragment, a F(ab')<sub>2</sub> fragment, a Fv fragment, a diabody, or a single chain antibody molecule.

34. The method of any preceding claim, wherein the antibody is coupled to a labeling  
5 group.

35. The method of claim 34, wherein the labeling group is an optical label, radioisotope, radionuclide, an enzymatic group, biotinyl group, a nucleic acid, oligonucleotide, enzyme, or a fluorescent label.

36. The method of claim 4, wherein the antibody is an anti-C1 complex antibody,  
10 optionally wherein the anti-C1 complex antibody inhibits C1r or C1s activation or prevents their ability to act on C2 or C4.

37. The method of claim 36, wherein the anti-C1 complex antibody binds to a combinatorial epitope within the C1 complex, wherein said combinatorial epitope comprises amino acids of both C1q and C1s; both C1q and C1r; both C1r and C1s; or each of C1q, C1r,  
15 and C1s.

38. The method of any one of claims 4- 37, wherein the antibody inhibits cleavage of C4 and does not inhibit cleavage of C2.

39. The method of any one of claims 4- 37, wherein the antibody inhibits cleavage of C2 and does not inhibit cleavage of C4.

20 40. The method of any one of claims 4-39, wherein the antibody binds mammalian C1q, C1r, or C1s.

41. The method of claim 40, wherein the antibody binds human C1q, C1r, or C1s.

42. The method of any one of claims 4-35, wherein the antibody binds mammalian C1 complex.

25 43. The method of any one of claims 4-42, wherein the antibody crosses the blood brain barrier.

44. The method of claim 43, wherein the antibody is covalently linked to a therapeutic agent.

45. The method of claim 44, wherein the therapeutic agent comprises an anti-inflammatory protein, neurotherapeutic agent, anti-viral, anti-parasitic, anti-bacterial,  
5 endocrine drug, metabolic drug, mitotoxin, chemotherapy drug, or siRNA.

46. The method of claim 45, wherein the neurotherapeutic agent is a neurotrophin selected from brain derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin-4/5, fibroblast growth factor (FGF)-2 and other FGFs, neurotrophin (NT)-3, erythropoietin (EPO), hepatocyte growth factor (HGF), epidermal growth factor (EGF),  
10 transforming growth factor (TGF)- $\alpha$ , TGF- $\beta$ , vascular endothelial growth factor (VEGF), interleukin-1 receptor antagonist (IL-1ra), ciliary neurotrophic factor (CNTF), glial-derived neurotrophic factor (GDNF), neurturin, platelet-derived growth factor (PDGF), heregulin, neuregulin, artemin, persephin, interleukins, granulocyte-colony stimulating factor (CSF), granulocyte-macrophage-CSF, netrins, cardiotrophin-1, hedgehogs, leukemia inhibitory  
15 factor (LIF), midkine, pleiotrophin, bone morphogenetic proteins (BMPs), saposins, semaphorins, or stem cell factor (SCF).

47. The method of any one of claims 4-45, wherein the antibody is a bispecific antibody recognizing a first antigen and a second antigen.

48. The method of claim 47, wherein the second antigen is transferrin receptor (TR),  
20 insulin receptor (HIR), insulin 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, or ANG1005.

49. The method of any preceding claim, wherein the antibody inhibits the classical  
25 complement activation pathway by an amount that ranges from at least 30% to at least 99.9%.

50. The method of any preceding claim, wherein the antibody inhibits the alternative complement activation pathway initiated by C1q binding.

51. The method of any preceding claim, wherein the antibody inhibits the alternative complement activation pathway by an amount that ranges from at least 30% to at least 99.9%.

52. The method of any preceding claim, wherein the antibody inhibits complement-dependent cell-mediated cytotoxicity (CDCC).

53. The method of claim 52, wherein the antibody inhibits complement-dependent cell mediated cytotoxicity (CDCC) activation pathway by an amount that ranges from at least  
5 30% to at least 99.9%.

54. The method of claim 52 or 53, wherein the antibody inhibits autoantibody and complement-dependent cell-mediated cytotoxicity (CDCC).

55. The method of any preceding claim, further comprising administering a second antibody selected from an anti-C1q antibody, an anti-C1r antibody, and an anti-C1s antibody.

10 56. The method of any preceding claim, further comprising administering to the subject a therapeutically effective amount of an inhibitor of antibody-dependent cellular cytotoxicity (ADCC).

57. The method of any preceding claim, further comprising administering to the subject a therapeutically effective amount of an inhibitor of the classical complement activation  
15 pathway.

58. The method of any preceding claim, further comprising administering to the subject a therapeutically effective amount of an inhibitor of the alternative complement activation pathway.

59. The method of any preceding claim, further comprising administering to the subject a  
20 therapeutically effective amount of an inhibitor of an interaction between the autoantibody and its corresponding autoantigen.

60. A method of determining a subject's risk of developing frontotemporal dementia, comprising:

- 25 (a) administering an anti-C1q, anti-C1r, or anti-C1s antibody to the subject, wherein the anti-C1q, anti-C1r, or anti-C1s antibody is coupled to a detectable label;
- (b) detecting the detectable label to measure the amount or location of C1q, C1r, or C1s in the subject; and

- (c) comparing the amount or location of one or more of C1q, C1r, or C1s to a reference, wherein the risk of developing frontotemporal dementia is characterized based on the comparison of the amount or location of one or more of C1q, C1r, or C1s to the reference.

- 5 61. The method of claim 60, wherein the detectable label comprises a nucleic acid, oligonucleotide, enzyme, radioactive isotope, biotin or a fluorescent label.
62. The method of claim 60, wherein the detectable label is detected using an imaging agent for x-ray, CT, MRI, ultrasound, PET and SPECT.
63. The method of claim 60, where in the fluorescent label is selected from fluorescein,  
10 rhodamine, cyanine dyes or BODIPY.
64. A kit comprising an antibody of any one of claims 5, 12, or 19, and a package insert comprising instructions for using the antibody to treat or prevent frontotemporal dementia.
65. A method of inhibiting synapse loss in a patient suffering frontotemporal dementia, comprising administering an antibody as defined in any one of claims 4-45.
- 15 66. The method of claim 65, wherein the frontotemporal dementia is associated with loss of synapses or loss nerve connections.
67. The method of claim 65 or claim 66, wherein the frontotemporal dementia is associated with synapse loss that is dependent on the complement receptor 3(CR3)/C3 or complement receptor CR1.
- 20 68. The method of any preceding claim, wherein the frontotemporal dementia is associated with pathological activity-dependent synaptic pruning.
69. The method of any preceding claim, wherein the frontotemporal dementia is associated with synapse phagocytosis by microglia.

Figure 1

EPTMYGEILSPNYFPQAYPSEVEKSWDIEVFEGYGIHLYFTHLDIELSENCAVDSVQIISG  
DTEEGELCGQRSSINPHSPIVEEFQVPYNKLQVIFKSDFSNEERFTGFAAYYVATDINEC  
TDFVDVPCSHFCNNFIGGYFCSCPFEYFLHDDMKNCGVNCSGDVFTALIGEIASPNYPKP  
YPENSRCYQIRLEKGFQVVVTLEREDFDVEAADSAGNCLDSLVPVAGDRQFGFYCGHGF  
PGPLNIETKSNALDIIFQTDLTGQKKGWKLRYHGDPMPCPKEDTFNSVWEPAAKAYVFRD  
VVQITCLDGFVVEGRVGATSFYSTCQSNKGWSNSKLKCQFVDCGIFESIENGKVEDPES  
TLFGSVIRYTCEEPPYYMENGSGGEYHCAGNGSWVNEVLGPPELPCVPCVGVPREPFEEK  
QRIIGSSDADIKNFPWQVFFDNFWAGGALINEYWVLTAHVVEGNREFTMYVGSTSVQTS  
RLAKSKMLTFEHVFIHFGWKLLLEVPEGRTNFDNDIALVBLKDPVKMGFTVSPICLPGTSS  
DYNLMGGDLGLISGWGRTEKRDRAVRLKAARLPVAFRLKCKEVKVEKPTADAEAYVFTPN  
MICAGGEKGMDSCKGDSGAFVQDPNDKTKFYAAGLVSWGPQCGTYGLYTRVKNYVDWI  
MKTMQENSTPRED (SEQ ID NO.70)

Figure 2

VH variant 1

102030405060708090100

GAAGTGGATGCTGGTGGAGTCTGGGGGAAGGCTTAAATGAAGGCTGGGAAGGCTGCTGAGACTGTCTGTGGCAGGCTCTGGATTCACTTTTCAGTAAATTATGCCA

E V M L V E S G D G L V N P S G S L R L S C A A S G F T F S N Y A

102030

110120130140150160170180190200

TGTCTTGGGTTTGGCAGGCTTCGAGGGAAGGGGCTGGAGTGGGCTGGCAACCATTTAGTAGTGGTGGTAGTGCACACCTATTATTATTAGACAGTGTGGAAGGCTTC

K S W V R Q A P G K G L R W V A T I S S G S S H T Y Y L D S V N G R

405052 A60

210220230240250260270280290300

ATTCACTATCTTCAGAGACAAATTCGAGGACAACTCTGTACCTTGCAGATGAGCTCTGAGGCTTGAGGAGACAGGGGCTTATTATTGTGCAAGGCTGTTT

F T I S R D N S K D T L F L Q N S S L R A R S F A L Y Y C A R L F

708082 A B C90

310320330340350

ACCGCTATGCTATGCACTATTGGGCTGAGGAGAGGCTCAGTCAAGGCTCTGCTCA

T G Y A M D Y W Q Q G T S V T V S S

100 A110

(SEQ ID NO.79)

Figure 3

VH variant 2

10 20 30 40 50 60 70 80 90  
GAAGTGTATCTCTGTGGAGCTCTGGGGGAGGCTTATGTAAGGCTCTGGAGGCTCTCTGAGCTCTCTCTGTCAGGCTCTCTGGATTCACCTTTCAATTAAGCCA  
E V S L V E S G G G L V K P G G S L R L S C A A G G F T F S N Y A  
10 20 30  
110 120 130 140 150 160 170 180 190 200  
TGTCTCTGGCTTCTGGCCAGGCTCTCAGGGAAGGGGCTGGATGTGGCTTGGCAACCAATTAGTAAGTGGTGGTACTGCACACCTATTATTTAGACACTGTGAGCGGTGG  
M S S V R Q A P C K G L E W V A T I S S G G S H T Y Y L D S V K G A  
40 50 52 A 60  
210 220 230 240 250 260 270 280 290 300  
ATTCACCATCTCCAGAGACAAATCCAAAGGACACCCCTGTATCTGTCAAATGAACACTCTNSAGGGCTGAGGAGCAAGGGGGCTGTATTATTSTCCAAAGACTGTTT  
P T L S R D M S E D T L Y L Q M S S L R A R D T A L Y Y C A R L F  
70 80 82 A B C 90  
310 320 330 340 350  
ACCGGCTATGCTATGGGACTATTTGGGCTCAAGGAACCCCTGGTCAACCGTCTCTCTCA  
T G Y A M D Y W G Q G T L V T V S S  
100 A 110

(SEQ ID NO.80)

Figure 4

VH variant 3

102030405060708090100

GAAGTCAATACCTGGGTGGAGTCTGGCGGAGCGTTAGTCAAGGCTGGAGGCTGGAGAGTCTCTGGTGGCAGCCCTCTGGATTCACTTCAGTAATTATGCGCA

E V M L V R S G G G L V R P G G S L R L S C A A S G F T F S N Y A

102030

110120130140150160170180190

TGTCCTTGGCTTCGCGCGCTCCAGTGGAGCGGCTGGAGTGGCTCCAAACCATTAAGTAGCTGGTGGTAGTCCACACCTATTATTAGACAGTGTGAAGGCTCG

M S W V D Q A P G K G L E W V A T I S S G G S H T Y Y L D S V E G R

40505260

210220230240250260270280290300

ATTCACCATCTCCAGAGACAAATTCCAAGACACCCCTGTACCTGCAAATGGAACAGTCTGAGGGCTGAGGACACCGCCCTGTATTATTGTGCAGAGACTGTTT

F T I S P D N S K N T L Y L Q M N S L R A E D T A L Y Y C A R L F

7080828486

310320330340350

ACCGGCTATGCTATGGACTATTGGGGTCAAGGAAACCCCTGGTTCACCGGCTCTCTCA

T G Y A M D Y W S Q G T L V T V S S

100110

(SEQ ID NO.81)

Figure 5

### VII variant 4

10 20 30 40 50 60 70 80 90 100  
CAAGTCAGCTTCGTCGAGTCTCGCGGACGCTTACGCGACCCGCGACGGGTCCTCGAGACTCTCTCTCTGACGCTCTCGAGTTCAGCTTTCAGTAAATTATGCGA  
E V Q L V E S S S S L V K F S G S L R L S C A A S G F T F S N Y A  
10 20 30  
110 120 130 140 150 160 170 180 190 200  
TGTCTTGGGCTTCGCGAGGTTCCAGGCGACGGGCTCGAGCTGCGTGGTAAACCAFTAGTAGTGGTGGTAGTCACACCTATTATTATTTAGACAGCTTTCGAGCGGTTC  
M S W V R Q A P S K G L E W V A T I S S G G S R T Y Y L D S V N G R  
40 50 60  
210 220 230 240 250 260 270 280 290 300  
ATTCACCATCTCCAGAGACCAATTCGCAAGAACACCCGTCGTAAGTGCAGATGAGACAGTCTGACGGGCTGAGGGGACAGCGCCCTGTATTATTTCTGCAAGACTTCTTT  
P T I S R D R S K R T L V L Q R D S L R A R D T A L Y Y C A R L F  
70 80 90  
310 320 330 340 350  
ACCGGCTATGCTATGGACTATTCGGGCTCGAGGAAACCCGTCGACCGTCTCTCTCA  
T G Y A M D Y W G Q G T L V T V S S  
100 110  
(SEQ ID NO.82)

Figure 6

VK variant 3

[illegible]

Figure 7

VK variant 2

[illegible]

Figure 8

VK variant 3

102030405060708090100

CAAAATTGTTCTCAGCCCBGTCTCCAGGCAACCTGTCTCTCTCTCCAGGGGAAACGGCCACCCCTGTCTTGCACAGCCAGCTCAAGTGTAAAGTTCCAGTTACT

Q I V L T Q S P A T L S L S P G R R A T L S C T A S S S V S S S Y

102030

110120130140150160170180190200

TGCACCTGCTGCGAGAGGACAGGAAAGACCCGACCAACTCTGGATTTATAGGCACATGCCAACCTGGCTTCTTGAATGCCATCTTGGCTTCACTGGACGAGTGG

L R R Y Q Q K F G R A P R L R I Y S T G R L A S G V P S R P S G S G

405060

210220230240250260270280290300

GTCTGGGAGCGATTCACCTTCCAGCAATCCAGCAGCCCTGCGAGCCCTGAAGATTTTGGCACTTATTAATGTCACACCACTATTATCGTTTACCAACCCATCAGCTTC

S G T D Y T L T I S S L Q P R D F A T Y Y C R Q Y Y R L P P I T F

70809095

310320

GCTCAGGCGGACCAACCTGGAGGTCAAA

G Q G T S L R I K

100105

(SEQ ID NO.85)

Figure 9

Table 3: VH Variants

| Amino Acid Position | IPN003 (Parental antibody) | VH Variant 1 | VH Variant 2 | VH Variant 3 | VH Variant 4 |
|---------------------|----------------------------|--------------|--------------|--------------|--------------|
| 3                   | M                          | M            | M            | M            | Q            |
| 10                  | A                          | G            | G            | G            | G            |
| 19                  | K                          | R            | R            | R            | R            |
| 40                  | I                          | A            | A            | A            | A            |
| 42                  | E                          | G            | G            | G            | G            |
| 44                  | R                          | G            | G            | G            | G            |
| 74                  | A                          | S            | S            | S            | S            |
| 75                  | R                          | K            | K            | K            | K            |
| 76                  | D                          | D            | D            | N            | N            |
| 82A                 | S                          | S            | N            | N            | N            |
| 84                  | S                          | A            | A            | A            | A            |
| 108                 | S                          | S            | L            | L            | L            |

Table 4: Vk Variants

| Amino Acid Position | IPN003 (Parental antibody) | Vk Variant 1 | Vk Variant 2 | Vk Variant 3 |
|---------------------|----------------------------|--------------|--------------|--------------|
| 10                  | I                          | I            | T            | T            |
| 11                  | M                          | L            | L            | L            |
| 13                  | A                          | L            | L            | L            |
| 15                  | L                          | P            | P            | P            |
| 19                  | V                          | A            | A            | A            |
| 21                  | M                          | M            | M            | L            |
| 22                  | T                          | S            | S            | S            |
| 42                  | S                          | K            | K            | K            |
| 43                  | S                          | A            | A            | A            |
| 60                  | A                          | S            | S            | S            |
| 70                  | F                          | F            | D            | D            |
| 72                  | S                          | T            | T            | T            |
| 78                  | M                          | L            | L            | L            |
| 79                  | E                          | Q            | Q            | Q            |
| 80                  | A                          | A            | P            | P            |
| 83                  | D                          | F            | F            | F            |
| 100                 | A                          | Q            | Q            | Q            |
| 106                 | L                          | I            | I            | I            |