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FIELD OF THE INVENTION

The present invention relates to the fields of immunology and molecular biology. More particularly, the present invention relates to monoclonal antibodies and compositions comprising the same for inhibiting the activation of the classical and lectin pathways of the complement system and use thereof in the treatment of human conditions. The invention in particular relates to monoclonal antibodies or antigen-binding fragments thereof that bind to human complement factor C2 and methods of making and using same.

BACKGROUND OF THE INVENTION

The complement system involves a cascading series of plasma enzymes, regulatory proteins, and proteins capable of cell lysis. Prior to activation, various complement factors circulate as inactive precursor proteins. Activation of the system leads to an activation cascade where one factor activates the subsequent one by specific proteolysis of complement protein further downstream in the cascade.

Activation of the complement system can occur via three pathways, the classical (or classic) pathway, the alternative pathway, and the lectin pathway. The classical pathway is activated by interaction of antigen and IgM, IgG1, IgG2, or IgG3 antibody to form immune complexes that bind C1q, a subunit of complement component C1. The alternative pathway is activated by IgA-containing immune complexes or recognition of bacteria and other activating surfaces. The lectin pathway is responsible for an antibody-independent pathway of complement activation that is initiated by binding of mannan-binding lectin (MBL), also known as mannose-binding lectin or mannan-binding protein (MBP), to certain carbohydrates on the surface of a variety of pathogens.

Activation of the classical pathway begins with sequential activation of C1, C4, and C2; C2 is in turn cleaved into C2a and C2b. Activation of the alternative pathway begins with sequential activation of complement components D, C3, and B. Each pathway cleaves and activates a common central component, C3 or the third complement factor, which results in the activation of a common terminal pathway leading to the formation of the membrane-attack complex (MAC, comprising complement components C5b-9; Muller-Eberhard, *Annu Rev Biochem* 1988, 57:321). During complement activation, several inflammatory peptides like the anaphylatoxins C3a and C5a are generated as well as the

MAC. These activation products elicit pleiotropic biological effects such as chemotaxis of leukocytes, degranulation of phagocytic cells, mast cells and basophils, smooth muscle contraction, increase of vascular permeability, and lysis of cells (Hugh, *Complement* 1986, 3:111). Complement activation products also induce the generation of toxic oxygen radicals and the synthesis and release of arachidonic acid metabolites and cytokines, in particular by phagocytes, which further amplifies the inflammatory response.

Although complement is an important line of defense against pathogenic organisms, its activation can also confer damage to otherwise healthy host cells. Inhibition of complement activation is therefore thought to be beneficial in treating and preventing complement-mediated tissue damage. Accordingly, there remains an urgent need in the art for novel therapeutic agents that inhibit one or more key components of the complement cascade.

SUMMARY OF THE INVENTION

Provided are novel monoclonal anti-human C2b antibodies and antigen-binding fragments thereof with improved features over existing antibodies, such as those described in WO2014/189378. A feature of the novel antibodies is the deletion of a glycosylation site in framework region 3 (FR3) of the heavy chain variable domain (VH). Notably, the novel antibodies provide improved homogeneity and therefore improved manufacturability, as well as unexpectedly improved functional properties, compared to existing antibodies. The improved functional properties include, for example, increased pI and enhanced potential for so-called antigen sweeping. The antibodies and antigen-binding fragments thereof will find use in human therapy.

An aspect of the invention is a monoclonal antibody or antigen-binding fragment thereof that specifically binds to human complement factor C2, wherein said monoclonal antibody or the antigen-binding fragment thereof comprises:

a VH domain comprising the amino acid sequence set forth in SEQ ID NO: 3; and

a VL domain comprising the amino acid sequence set forth in SEQ ID NO: 2.

An aspect of the invention is a pharmaceutical composition comprising the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention, and a pharmaceutically acceptable carrier.

An aspect of the invention is a nucleic acid molecule or plurality of nucleic acid molecules encoding the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention.

An aspect of the invention is a vector or plurality of vectors comprising the nucleic acid molecule or the plurality of nucleic acid molecules in accordance with the invention.

An aspect of the invention is a host cell comprising a nucleic acid molecule or plurality of nucleic acid molecules encoding the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention.

An aspect of the invention is a host cell comprising a vector or plurality of vectors comprising the nucleic acid molecule or the plurality of nucleic acid molecules in accordance with the invention.

An aspect of the invention is a method of making a monoclonal antibody or antigen-binding fragment thereof in accordance with the invention, the method comprising culturing a population of cells according to the invention under conditions suitable for expression of the monoclonal antibody or antigen-binding fragment thereof; and isolating the monoclonal antibody or antigen-binding fragment from the cells.

An aspect of the invention is a monoclonal antibody, antigen-binding fragment thereof ,or a pharmaceutical composition of the invention for use in a method of inhibiting activation of the classical or lectin pathway in a subject and treating or preventing a disease or condition selected from experimental allergic neuritis, type II collagen-induced arthritis, myasthenia gravis, hemolytic anemia, glomerulonephritis, idiopathic membranous nephropathy, rheumatoid arthritis, systemic lupus erythematosus, immune complex-induced vasculitis, multiple sclerosis, adult respiratory distress syndrome, stroke, xenotransplantation, allotransplantation, burn injuries, sepsis, septic shock, toxicity induced by the in vivo administration of cytokines or mAbs, antibody-mediated rejection of allografts such as kidney allografts, multiple trauma, ischemia-reperfusion injuries, and myocardial infarction.

The following embodiments apply to all aspects of the invention.

In certain embodiments, the monoclonal antibody comprises a full-length monoclonal antibody.

In certain embodiments, the monoclonal antibody comprises a human IgG heavy chain constant domain.

In certain embodiments, the heavy chain constant domain comprises a human IgG1 heavy chain constant domain. In certain embodiments, the human IgG1 heavy chain constant domain comprises the amino acid sequence set forth in SEQ ID NO: 4.

In certain embodiments, the heavy chain constant domain comprises a human IgG4 heavy chain constant domain. In some embodiments, the human IgG4 heavy chain constant domain comprises the amino acid sequence set forth in SEQ ID NO: 5.

In certain embodiments, the monoclonal antibody comprises a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 6 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 7.

In certain embodiments, the monoclonal antibody comprises a heavy chain comprising the amino acid sequence set forth as SEQ ID NO: 8 and a light chain comprising the amino acid sequence set forth as SEQ ID NO: 7.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 depicts a polyacrylamide gel loaded with indicated samples. Larger molecular weight bands for samples in lanes 4, 5, 8, and 9 (arrows) show band splitting and shifting for antibodies with VH3 and VH4.

Fig. 2 is a graph depicting total levels of indicated antibodies over the course of 31 days in cynomolgus monkeys. The following antibodies were tested: BRO2-glyc-IgG4 (monkeys 1 and 2, glycosylated VH) and BRO2-IgG4 (monkeys 5 and 6, non-glycosylated VH).

Figs. 3A-3I are graphs depicting levels of free C2 (plotted as OD 450 nm over time) in serum over the course of 31 days from administration of various monoclonal antibodies to cynomolgus monkeys. The following antibodies were tested: BRO2-glyc-IgG4 (Fig. 3A; monkeys 1 and 2), negative control (Fig. 3B; monkeys 3 and 4), BRO2-IgG4 (Fig. 3C; monkeys 5 and 6), BRO2-IgG4-NH (Fig. 3D; monkeys 7 and 8), BRO2-IgG1-LALA-NH (Fig. 3E; ARGX-117; monkeys 9 and 10), His1-IgG4 (Fig. 3F; monkeys 11 and 12), His1-IgG4-NH (Fig. 3G; monkeys 13 and 14), His1-IgG1-LALA-NH (Fig. 3H; monkeys 15 and 16), and His2-IgG4 (Fig. 3I; monkeys 17 and 18).

Fig. 4 is a graph depicting average free C2 levels (plotted as OD 450 nm over time) in serum over the course of 31 days from cynomolgus monkeys administered various indicated monoclonal antibodies.

Fig. 5 is a graph depicting free C2 levels (plotted as OD 450 nm over time) in serum of cynomolgus monkeys treated with indicated non-glycosylated antibodies.

Figs. 6A-6D are a series of graphs depicting free C2 levels (plotted as OD 450 nm) in cynomolgus monkeys as determined at indicated times prior to or following administration of antibodies. Monkeys are as in Figs. 3A-3I. Fig. 6A, pre versus pre plus 500 mg/ml BRO-2; Fig. 6B, 4 hours versus 1 day; Fig. 6C, 4 hours versus 2 days; Fig. 6D, day 11 versus day 27. ADA, anti-drug antibody.

Figs. 7A-7P are a series of graphs depicting immunogenicity (plotted as OD 450 nm) over 30 days of anti-C2 antibodies or negative control monoclonal antibody administered to cynomolgus monkeys. Monkeys are as in Figs. 3A-3I. Fig. 7A, monkey 1; Fig. 7B, monkey 2; Fig. 7C, monkey 5; Fig. 7D, monkey 6; Fig. 7E, monkey 7; Fig. 7F, monkey 8; Fig. 7G, monkey 9; Fig. 7H, monkey 10; Fig. 7I, monkey 11; Fig. 7J, monkey 12; Fig. 7K, monkey 13; Fig. 7L, monkey 14; Fig. 7M, monkey 15; Fig. 7N, monkey 16; Fig. 7O, monkey 17; Fig. 7P, monkey 18.

Figs. 8A-8F are a series of graphs depicting immunogenicity (plotted as OD 450 nm over time) over 60 days of anti-C2 monoclonal antibodies administered to cynomolgus monkeys. Monkeys are as in Figs. 3A-3I. Fig. 8A, monkey 5; Fig. 8B, monkey 6; Fig. 8C, monkey 9; Fig. 8D, monkey 10; Fig. 8E, monkey 15; Fig. 8F, monkey 16. ADA, anti-drug antibody.

Figs. 9A-9D depict ARGX-117 binding to C2 assessed by Western blot analysis and surface plasmon resonance (SPR). Fig. 9A depicts Western blot analysis of serum with ARGX-117 (representative result): Lane 1: MW size marker; Lane 2: recombinant human C2 control (size about 100 kDa); Lane 3: serum; Lane 4: induction of complement activation by addition of aggregated IgG to serum and incubation at 37°C; Lane 5: C2-deficient serum.

Fig. 9B depicts SPR analysis with C2 immobilized on chip and different ARGX-117 Fabs as eluate.

Fig. 9C depicts SPR analysis with biotin-C4b immobilized to streptavidin-chip and human C2 with and without mAbs as eluate; black: no pre-incubation; grey: anti-FXI; control human IgG4 mAb; turquoise: non-inhibitory anti-C2 clone anti-C2-63, i.e., clone 63 recognizing the large subunit of C2 (C2a); red: ARGX-117; all at 5 to 1 molar ratios; curves were normalized to signal just before the injection of C2 on the C4b chips.

Fig. 9D depicts SPR analysis with biotin-C4b immobilized to streptavidin-chip and consecutively human C2 and mAbs as eluate; black: running buffer; grey: anti-FXI; control human IgG4 mAb; turquoise: non-inhibitory anti-C2 clone anti-C2-63; red: ARGX-117; curves were normalized just before the addition of the mAbs.

Fig. 10 depicts a schematic representation of domain swap mutants between C2 (SEQ ID NO: 21) and complement Factor B (FB) (SEQ ID NO: 50). In both proteins the small fragment (C2b in complement C2; SEQ ID NO: 44 or FBa in complement Factor B; SEQ ID NO: 51) consists of three Sushi (or complement control protein (CCP)) domains, whereas the large fragment is composed of a von Willebrand Factor type A (VWFA) domain and a peptidase S1 domain. Note that the sequences in between the individual domains were not taken along in these mutants but may also consist of epitopes. Additional sequences include C2a, SEQ ID NO: 43; C2b S1, SEQ ID NO: 45; C2b S2, SEQ ID NO: 46; C2b S3, SEQ ID NO: 47; C2 VWFA, SEQ ID NO: 48; C2 peptidase S1, SEQ ID NO: 49; FBb, SEQ ID NO: 52; FBa S1, SEQ ID NO: 53; FBa S2, SEQ ID NO: 54; FBa S3, SEQ ID NO: 55; FB VWFA, SEQ ID NO: 56; and FB peptidase 1, SEQ ID NO: 57.

Fig. 11 depicts results obtained with an anti-FLAG ELISA performed on domain-swap mutants. Five-times diluted supernatants from transfected HEK293 cells were used for coating, and anti-FLAG mouse monoclonal Ab in combination with HRP-labeled anti-mouse IgG were used for detection.

Fig. 12 depicts results obtained with a domain swap ELISA performed with anti-C2-5F2.4. Anti-C2-5F2.4 mAb (human IgG4 S241P VH4/VL3 LC-13/03-163A Bioceros) was used for coating, plates were incubated with 20 times diluted supernatant of HEK293 transfectants, and binding was detected by an anti-FLAG Ab. Representative results from two independent experiments with similar outcome.

Fig. 13 depicts an amino acid sequence alignment of human and mouse Sushi 2 (S2) domain of C2b. Human S2, SEQ ID NO: 46; Mouse S2, SEQ ID NO: 58. Stars indicate sequence identity.

Fig. 14 depicts results obtained with an anti-FLAG ELISA on fine mapping mutants. Undiluted supernatants from transfected HEK293 cells were used for coating, and biotin-labeled anti-FLAG mouse monoclonal Ab in combination with HRP-labeled SA conjugate were used for detection.

Fig. 15 depicts results on fine mapping mutants. Anti-C2-5F2.4 mAb (human IgG4 S241P VH4/VL3 LC-13/03-163A Bioceros) was used for coating, plates were incubated

with 20 times diluted supernatant of HEK293 transfectants, and binding was detected by an anti-FLAG Ab.

Fig. 16 depicts a plan of cluster mapping mutants using three amino acid mutations for each cluster, locations for which indicated with bold font in the human sequence. Each human sequence was mutated to substitute the corresponding mouse amino acid for the human amino acid shown in bold. Human S2, SEQ ID NO: 46; Mouse S2, SEQ ID NO: 58. Stars indicate sequence identity.

Figs. 17A and 17B depict anti-FLAG ELISA on cluster mapping mutants. Fig. 17A depicts five-times diluted supernatants from transfected HEK293 cells were used for coating and anti-FLAG mouse monoclonal Ab in combination with HRP-labeled anti-mouse IgG as detection. GFP, green fluorescent protein.

Fig. 17B depicts anti-C2-5F2.4 binding to cluster mutants. Anti-C2-5F2.4 mAb (human IgG4 S241P VH4/VL3, LC-13/03-163A, Bioceros) was used as coat, plates were incubated with 20-times diluted supernatant of HEK293 transfectants, and binding was detected by an anti-FLAG Ab. GFP, green fluorescent protein.

DETAILED DESCRIPTION

Definitions

"Antibody" or "Immunoglobulin" – As used herein, the term "immunoglobulin" includes a polypeptide having a combination of two heavy and two light chains whether or not it possesses any relevant specific immunoreactivity. As used herein, the term "antibody" refers to such assemblies which have significant specific immunoreactive activity to an antigen of interest (e.g. the complex of complement proteins including C2). The term "C2 antibodies" is used herein to refer to antibodies which exhibit immunological specificity for the complex of complement proteins including C2, particularly the human C2 protein and the domains which are formed through cleavage of C2, and in some cases species homologues thereof. Antibodies and immunoglobulins comprise light and heavy chains, with or without an interchain covalent linkage between them. Basic immunoglobulin structures in vertebrate systems are relatively well understood.

Five distinct classes of antibody (IgG, IgM, IgA, IgD, and IgE) can be distinguished biochemically. All five classes of antibodies are within the scope of the present invention. The following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, immunoglobulins typically comprise two identical light

polypeptide chains of molecular weight approximately 23,000 Daltons, and two identical heavy chains of molecular weight 53,000-70,000. The four chains are joined by disulfide bonds in a "Y" configuration wherein the light chains bracket the heavy chains starting at the mouth of the "Y" and continuing through the variable region.

The light chains of an antibody are classified as either kappa (κ) or lambda (λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the "tail" portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon, (γ, μ, α, δ, or ε) with some subclasses among them (e.g., γ1-γ4). It is the nature of this chain that determines the "class" of the antibody as IgG, IgM, IgA, IgD or IgE, respectively. The immunoglobulin subclasses (isotypes) e.g., IgG1, IgG2, IgG3, IgG4, IgA1, etc., are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernible to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant invention.

As indicated above, the variable region of an antibody allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VL domain and VH domain of an antibody combine to form a variable region that defines a three-dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three complementary determining regions (CDRs) on each of the VH and VL chains.

"Binding Molecule" – As used herein, the term "binding molecule" is a generic term intended to encompass the antibodies and antigen-binding fragments thereof in accordance with the present disclosure.

"Binding Site" – As used herein, the term "binding site" comprises a region of a polypeptide which is responsible for selectively binding to a target antigen of interest. Binding domains comprise at least one binding site. Exemplary binding domains include an

antibody variable domain. The antibody molecules of the invention may comprise a single binding site or multiple (e.g., two, three or four) binding sites.

"Variable region" or "variable domain" – The term "variable" refers to the fact that certain portions of the variable domains VH and VL differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its target antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called "hypervariable loops" in each of the VL domain and the VH domain which form part of the antigen-binding site. The first, second and third hypervariable loops of the Vlambda light chain domain are referred to herein as L1(λ), L2(λ) and L3(λ) and may be defined as comprising residues 24-33 (L1(λ), consisting of 9, 10 or 11 amino acid residues), 49-53 (L2(λ), consisting of 3 residues) and 90-96 (L3(λ), consisting of 5 residues) in the VL domain (Morea et al., *Methods* 20:267-279 (2000)). The first, second and third hypervariable loops of the Vkappa light chain domain are referred to herein as L1(κ), L2(κ) and L3(κ) and may be defined as comprising residues 25-33 (L1(κ), consisting of 6, 7, 8, 11, 12 or 13 residues), 49-53 (L2(κ), consisting of 3 residues) and 90-97 (L3(κ), consisting of 6 residues) in the VL domain (Morea et al., *Methods* 20:267-279 (2000)). The first, second and third hypervariable loops of the VH domain are referred to herein as H1, H2 and H3 and may be defined as comprising residues 25-33 (H1, consisting of 7, 8 or 9 residues), 52-56 (H2, consisting of 3 or 4 residues) and 91-105 (H3, highly variable in length) in the VH domain (Morea et al., *Methods* 20:267-279 (2000)).

Unless otherwise indicated, the terms L1, L2 and L3 respectively refer to the first, second and third hypervariable loops of a VL domain, and encompass hypervariable loops obtained from both Vkappa and Vlambda isotypes. The terms H1, H2 and H3 respectively refer to the first, second and third hypervariable loops of the VH domain, and encompass hypervariable loops obtained from any of the known heavy chain isotypes, including γ, μ, α, δ or ε.

The hypervariable loops L1, L2, L3, H1, H2 and H3 may each comprise part of a "complementarity determining region" or "CDR", as defined below. The terms "hypervariable loop" and "complementarity determining region" are not strictly synonymous, since the hypervariable loops (HVs) are defined on the basis of structure, whereas complementarity determining regions (CDRs) are defined based on sequence variability (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public

Health Service, National Institutes of Health, Bethesda, MD., 1983) and the limits of the HVs and the CDRs may be different in some VH and VL domains.

The CDRs of the VL and VH domains can typically be defined as comprising the following amino acids: residues 24-34 (LCDR1), 50-56 (LCDR2) and 89-97 (LCDR3) in the light chain variable domain, and residues 31-35 or 31-35b (HCDR1), 50-65 (HCDR2) and 95-102 (HCDR3) in the heavy chain variable domain; (Kabat et al., *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)). Thus, the HVs may be comprised within the corresponding CDRs and references herein to the "hypervariable loops" of VH and VL domains should be interpreted as also encompassing the corresponding CDRs, and vice versa, unless otherwise indicated.

The more highly conserved portions of variable domains are called the framework region (FR), as defined below. The variable domains of native heavy and light chains each comprise four FRs (FR1, FR2, FR3 and FR4, respectively), largely adopting a β-sheet configuration, connected by the three hypervariable loops. The hypervariable loops in each chain are held together in close proximity by the FRs and, with the hypervariable loops from the other chain, contribute to the formation of the antigen-binding site of antibodies. Structural analysis of antibodies revealed the relationship between the sequence and the shape of the binding site formed by the complementarity determining regions (Chothia et al., *J. Mol. Biol.* 227: 799-817 (1992)); Tramontano et al., *J. Mol. Biol*, 215:175-182 (1990)). Despite their high sequence variability, five of the six loops adopt just a small repertoire of main-chain conformations, called "canonical structures". These conformations are first of all determined by the length of the loops and secondly by the presence of key residues at certain positions in the loops and in the framework regions that determine the conformation through their packing, hydrogen bonding or the ability to assume unusual main-chain conformations.

"Framework region" – The term "framework region" or "FR region" as used herein, includes the amino acid residues that are part of the variable region, but are not part of the CDRs (e.g., using the Kabat definition of CDRs). Therefore, a variable region framework is between about 100-120 amino acids in length but includes only those amino acids outside of the CDRs. For the specific example of a heavy chain variable domain and for the CDRs as defined by Kabat *et al.*, framework region 1 corresponds to the domain of the variable region encompassing amino acids 1-30; framework region 2 corresponds to the domain of

the variable region encompassing amino acids 36-49; framework region 3 corresponds to the domain of the variable region encompassing amino acids 66-94, and framework region 4 corresponds to the domain of the variable region from amino acids 103 to the end of the variable region. The framework regions for the light chain are similarly separated by each of the light chain variable region CDRs. Similarly, using the definition of CDRs by Chothia *et al.* or McCallum *et al.* the framework region boundaries are separated by the respective CDR termini as described above. In preferred embodiments the CDRs are as defined by Kabat.

In naturally occurring antibodies, the six CDRs present on each monomeric antibody are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding site as the antibody assumes its three-dimensional configuration in an aqueous environment. The remainder of the heavy and light variable domains show less inter-molecular variability in amino acid sequence and are termed the framework regions. The framework regions largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, these framework regions act to form a scaffold that provides for positioning the six CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding site formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to the immunoreactive antigen epitope. The position of CDRs can be readily identified by one of ordinary skill in the art.

"Non-glycosylated" – As used herein, the term "non-glycosylated" refers to a form of antibody or antigen-binding fragment thereof which lacks glycosylation at a potential glycosylation site in the antibody or antigen-binding fragment. In certain embodiments, the term "non-glycosylated" refers to a form of antibody or antigen-binding fragment thereof which lacks glycosylation at a potential N-linked glycosylation site in antibody or antigen-binding fragment. In certain embodiments, the term "non-glycosylated" refers to a form of antibody or antigen-binding fragment thereof which lacks glycosylation at a potential N-linked glycosylation site in the variable region of the heavy chain.

"Constant region" – As used herein, the term "constant region" refers to the portion of the antibody molecule outside of the variable domains or variable regions. Immunoglobulin light chains have a single domain "constant region", typically referred to as the "CL or CL1 domain". This domain lies C-terminal to the VL domain.

Immunoglobulin heavy chains differ in their constant region depending on the class of immunoglobulin (γ, μ, α, δ, ε). Heavy chains γ, α and δ have a constant region consisting of three immunoglobulin domains (referred to as CH1, CH2 and CH3) with a flexible hinge region separating the CH1 and CH2 domains. Heavy chains μ and ε have a constant region consisting of four domains (CH1-CH4). The constant domains of the heavy chain are positioned C-terminal to the VH domain.

The numbering of the amino acids in the heavy and light immunoglobulin chains run from the N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain. Different numbering schemes are used to define the constant domains of the immunoglobulin heavy and light chains. In accordance with the EU numbering scheme, the heavy chain constant domains of an IgG molecule are identified as follows: CH1 – amino acid residues 118-215; CH2 – amino acid residues 231-340; CH3 – amino acid residues 341-446. The "hinge region" includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux K.H. et al. *J. Immunol.* 161:4083-90 1998). Antibodies of the invention comprising a "fully human" hinge region may contain one of the hinge region sequences shown in **Table 1** below.

Table 1. Human hinge sequences

IgG	Upper hinge	Middle hinge	Lower hinge
IgG1	EPKSCDKTHT (SEQ ID NO: 9)	CPPCP (SEQ ID NO: 10)	APELLGGP (SEQ ID NO: 11)
IgG2	ERK (SEQ ID NO: 12)	CCVECPPPCP (SEQ ID NO: 13)	APPVAGP (SEQ ID NO: 14)
IgG3	ELKTPLGDTTHT (SEQ ID NO: 15)	CPRCP $(EPKSCDTPPPCPRCP)_3$ (SEQ ID NO: 16)	APELLGGP (SEQ ID NO: 17)
IgG4	ESKYGPP (SEQ ID NO: 18)	CPSCP (SEQ ID NO: 19)	APEFLGGP (SEQ ID NO: 20)

"Fragment" – The term "fragment", as used in the context of antibodies of the invention, refers to a part or portion of an antibody or antibody chain comprising fewer

amino acid residues than an intact or complete antibody or antibody chain. The term "antigen-binding fragment" refers to a polypeptide fragment of an immunoglobulin or antibody that specifically binds antigen or competes with intact antibody (i.e., with the intact antibody from which they were derived) for antigen-specific binding (e.g., specific binding to the C2 protein or to a portion thereof). As used herein, the term "fragment" of an antibody molecule includes antigen-binding fragments of antibodies, for example, an antibody light chain variable domain (VL), an antibody heavy chain variable domain (VH), a single chain antibody (scFv), a F(ab')2 fragment, a Fab fragment, an Fd fragment, an Fv fragment, a one-armed (monovalent) antibody, diabodies, triabodies, tetrabodies or any antigen-binding molecule formed by combination, assembly or conjugation of such antigen-binding fragments. The term "antigen-binding fragment" as used herein is further intended to encompass antibody fragments selected from the group consisting of unibodies, domain antibodies and nanobodies. Fragments can be obtained, e.g., via chemical or enzymatic treatment of an intact or complete antibody or antibody chain, or by recombinant means.

Complement Component C2

The second component of human complement (C2) is a 90-100 kDa glycoprotein which participates in the classical and lectin pathways of complement activation. C2 can be activated by C1s of the classical pathway or by activated MASP2 of the lectin pathway. C2 binds to surface-bound C4b (in the presence of Mg^{2+}) to form a C4bC2 complex, which then is cleaved by activated C1s or MASP2 into two fragments: a larger 70 kDa fragment, traditionally designated C2a, which remains attached to C4b to form a C3-convertase C4bC2a, and a smaller 30 kDa N-terminal fragment, traditionally designated C2b, which is released into the fluid phase. Some authors have recently reversed designations of C2a and C2b, such that C2b refers to the bigger 70 kDa fragment, and C2a refers to the smaller 30 kDa fragment. As used herein, C2a shall refer to the bigger 70 kDa fragment, and C2b shall refer to the smaller 30 kDa fragment. Once activated and bound to C4b, C2a constitutes the catalytic subunit of the C3 and C5 convertases which are able to cleave C3 and C5, respectively.

The amino acid sequence of human C2 is known (GenBank Accession No. NM_000063) and shown as SEQ ID NO: 21.

Amino Acid Sequence of human C2 (SEQ ID NO: 21):

MGPLMVLFCLLFLYPGLADSAPSCPQNVNISGGTFTLSHGWAPGSLLTYSCPQGLYPSPAS
RLCKSSGQWQTPGATRSLSKAVCKPVRCPAPVSFENGIYTPRLGSYPVGGNVSFECEDGFI
LRGSPVRQCRPNGMWDGETAVCDNGAGHCPNPGISLGAVRTGFRFGHGDKVRYRCSSNLVL
TGSSERECQGNGVWSGTEPICRQPYSYDFPEDVAPALGTSFSHMLGATNPTQKTKESLGRK
IQIQRSGHLNLYLLLDCSQSVSENDFLIFKESASLMVDRIFSFEINVSVAIITFASEPKVL
MSVLNDNSRDMTEVISSLENANYKDHENGTGTNTYAALNSVYLMMNNQMRLLGMETMAWQE
IRHAIILLTDGKSNMGGSPKTAVDHIREILNINQKRNDYLDIYAIGVGKLDVDWRELNELG
SKKDGERHAFILQDTKALHQVFEHMLDVSKLTDTICGVGNMSANASDQERTPWHVTIKPKS
QETCRGALISDQWVLTAAHCFRDGNDHSLWRVNVGDPKSQWGKEFLIEKAVISPGFDVFAK
KNQGILEFYGDDIALLKLAQKVKMSTHARPICLPCTMEANLALRRPQGSTCRDHENELLNK
QSVPAHFVALNGSKLNINLKMGVEWTSCAEVVSQEKTMFPNLTDVREVVTDQFLCSGTQED
ESPCKGESGGAVFLERRFRFFQVGLVSWGLYNPCLGSADKNSRKRAPRSKVPPPRDFHINL
FRMQPWLRQHLGDVLNFLPL

As with many other plasma proteins, C2 has a modular structure. Starting from its N-terminus, C2 consists of three complement control protein modules (CCP1-3, also known as short consensus repeats (SCR) or sushi-domain repeats), a von Willebrand factor type A (vWFA) domain containing a metal-ion-dependent adhesion site, and a serine protease (SP) domain (Arlaud et al., *Adv Immunol* 1998, 69: 249). Electron microscopy studies have revealed that C2 consists of three domains. The three CCP modules (CCP1-3) together form the N-terminal domain, which corresponds to C2b. The vWFA domain constitutes the second domain, and the SP domain makes up the third domain. The second and third domains together constitute the larger C2a portion of the molecule.

CCP modules are common structural motifs that occur in a number of proteins. These globular units consist of approximately 60 amino acid residues and are folded into a compact six- to eight-stranded β-sheet structure built around four invariant disulfide-bonded cysteine residues (Norman et al., *J Mol Biol* 1991, 219: 717). Neighboring CCP modules are covalently attached by poorly conserved linkers.

The initial binding of C2 to surface-bound C4b is mediated by two low-affinity sites, one on C2b (Xu & Volanakis, *J Immunol* 1997, 158: 5958) and the other on the vWFA domain of C2a (Horiuchi et al., *J Immunol* 1991, 47: 584). Though the crystal structure of C2b and C2a have been determined to 1.8 Å resolution (Milder et al., *Structure* 2006, 14: 1587; Krishnan et al., *J Mol Biol* 2007, 367: 224; Krishnan et al., *Acta Cristallogr D Biol Crystallogr* 2009, D65: 266), the exact topology and structure of the amino acid

residues constituting the contact site(s) for C4 and C3 on C2 are unknown. Thus the amino acid residues of C2 involved in the interaction with C4 remain to be established (Krishnan et al., *Acta Cristallogr D Biol. Crystallogr* 2009, D65: 266).

Anti-C2 Antibodies

Described herein is a monoclonal antibody or antigen-binding fragment thereof that specifically binds to human complement factor C2, wherein said monoclonal antibody or fragment thereof comprises:

a VH domain comprising the amino acid sequence set forth in SEQ ID NO: 1; and

a VL domain comprising the amino acid sequence set forth in SEQ ID NO: 2;

wherein amino acid residues 72-74 (Kabat numbering) of the VH domain consist of $X_1X_2X_3$, respectively, wherein X_2 is any amino acid, and $X_1X_2X_3$ is not NX_2S or NX_2T.

The VH domain comprises complementarity determining regions (CDRs) HCDR1, HCDR2, and HCDR3. The VL domain comprises CDRs LCDR1, LCDR2, and LCDR3. The amino acid sequences of HCDR1, HCDR2, HCDR3, LCDR1, LCDR2, and LCDR3 are shown in **Table 2**.

Table 2. CDRs

HCDR1	DYNMD	(SEQ ID NO: 22)
HCDR2	DINPNYESTGYNQKFKG	(SEQ ID NO: 23)
HCDR3	EDDHDAFAY	(SEQ ID NO: 24)
LCDR1	RASKSVRTSGYNYMH	(SEQ ID NO: 25)
LCDR2	LASNLKS	(SEQ ID NO: 26)
LCDR3	QHSRELPYT	(SEQ ID NO: 27)

In certain embodiments, the monoclonal antibody or antigen-binding fragment thereof specifically binds to human complement factor C2b. In certain embodiments, the monoclonal antibody or antigen-binding fragment thereof specifically binds to an epitope in a portion of human complement factor C2 corresponding to human complement factor C2b.

The variable domain of the heavy chain may be non-glycosylated. In accordance with the invention, the amino acid sequence of the variable domain of the heavy chain does not include a potential glycosylation site which is characterized by the sequence N-X-S/T,

where N represents asparagine, X represents any amino acid, and S/T represents serine or threonine. Antibodies with a VH domain comprising the sequence N-X-S/T can be modified so that these residues consist of $X_1X_2X_3$, respectively, wherein X_2 is any amino acid, and $X_1X_2X_3$ is not NX_2S or NX_2T. That is, X_1 can be any amino acid other than N, and/or X_3 can be any amino acid other than S or T. Antibodies with a VH domain comprising the sequence N-X-S or N-X-T can be modified so that these three residues consist of D-X-S, respectively. Antibodies with a VH domain comprising the sequence N-X-S or N-X-T can be modified so that these three residues consist of D-X-T, respectively.

Heavy chain amino acids at residues 72-74 (Kabat numbering) may consist of $X_1X_2X_3$, respectively, wherein X_2 is any amino acid, and $X_1X_2X_3$ is not NX_2S or NX_2T.

Heavy chain amino acids at residues 72-74 (Kabat numbering) may consist of DX_2S.

In accordance with the invention, heavy chain amino acids at residues 72-74 (Kabat numbering) consist of DKS.

In accordance with the claims, the VH domain comprises the amino acid sequence set forth in SEQ ID NO: 3.

In certain embodiments, the amino acid sequence of the VH domain consists of the sequence set forth in SEQ ID NO: 3.

In accordance with the claims, the VL domain comprises the amino acid sequence set forth in SEQ ID NO: 2.

In certain embodiments, the amino acid sequence of the VL domain consists of the sequence set forth in SEQ ID NO: 2.

In accordance with the claims, the VH domain comprises the amino acid sequence set forth in SEQ ID NO: 3, and the VL domain comprises the amino acid sequence set forth in SEQ ID NO: 2.

In certain embodiments, the amino acid sequence of the VH domain consists of the sequence set forth in SEQ ID NO: 3, and the amino acid sequence of the VL domain consists of the sequence set forth in SEQ ID NO: 2.

The amino acid sequences of SEQ ID NO: 3 and SEQ ID NO: 2 are shown in **Table 3**. SEQ ID NO: 2 corresponds to the VL (VK3) domain of humanized 5F2.4 (BRO2) disclosed in U.S. Patent No. 9,944,717 to Broteio Pharma B.V. Also shown in **Table 3**, SEQ ID NO: 28 corresponds to the VH (VH4) domain of humanized 5F2.4 (BRO2) disclosed in U.S. Patent No. 9,944,717.

Table 3. VH and VL Domains

ID	Sequence	SEQ ID NO:
5F2.4 VH4	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVNKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSS	28
VH4.2 generic	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTV$X_1X_2X_3$ISTAYMELSSLRSEDTAVYYCAR EDDHDAFAYWGQGTLVTVSS	1
VH4.2 ARGX-117	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSS	3
5F2.4 VK3	DNVLTQSPDSLAVSLGERATISCRASKSVRTSGYNYMHWYQQKPGQPPKL LIYLASNLKSGVPDRFSGSGSGTDFTLTISSLQAEDAATYYCQHSRELPY TFGQGTKLEIK	2

In certain embodiments, the monoclonal antibodies of the invention include the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human antibody, in particular human IgG1, IgG2, IgG3 or IgG4.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG1 and includes the substitutions L234A and L235A in the CH2 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG1 and includes the substitutions H433K and N434F in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG1 and includes the substitutions L234A and L235A in the CH2 domain, and the substitutions H433K and N434F in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4. In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitution S228P in the hinge domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitution L445P in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes both the substitution S228P in the hinge domain and the substitution L445P in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitutions H433K and N434F in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitution S228P in the hinge domain, and the substitutions H433K and N434F in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitutions H433K, N434F, and L445P in the CH3 domain.

In certain embodiments, the antibody includes the CH1 domain, hinge domain, CH2 domain, and CH3 domain of a human IgG4 and includes the substitution S228P in the hinge domain, and the substitutions H433K, N434F, and L445P in the CH3 domain.

In certain embodiments, the monoclonal antibody comprises a human IgG heavy chain constant domain. In certain embodiments, the heavy chain constant domain comprises a human IgG1 heavy chain constant domain. In certain embodiments, the heavy chain constant domain consists of a human IgG1 heavy chain constant domain.

In certain embodiments, the heavy chain constant domain comprises a human IgG1 heavy chain constant domain comprising the amino acid sequence set forth as SEQ ID NO: 29. In certain embodiments, the amino acid sequence of the heavy chain constant domain consists of the sequence set forth as SEQ ID NO: 29.

In certain embodiments, the heavy chain constant domain comprises a human IgG1 heavy chain constant domain comprising the amino acid sequence set forth as SEQ ID NO: 4. In certain embodiments, the amino acid sequence of the heavy chain constant domain consists of the sequence set forth as SEQ ID NO: 4.

In certain embodiments, the heavy chain constant domain comprises a human IgG4 heavy chain constant domain. In certain embodiments, the heavy chain constant domain consists of a human IgG4 heavy chain constant domain.

In certain embodiments, the heavy chain constant domain comprises a human IgG4 heavy chain constant domain comprising the amino acid sequence set forth as SEQ ID NO:

30. In certain embodiments, the amino acid sequence of the heavy chain constant domain consists of the sequence set forth as SEQ ID NO: 30.

In certain embodiments, the heavy chain constant domain comprises a human IgG4 heavy chain constant domain comprising the amino acid sequence set forth as SEQ ID NO: 31. In certain embodiments, the amino acid sequence of the heavy chain constant domain consists of the sequence set forth as SEQ ID NO: 31.

In certain embodiments, the heavy chain constant domain comprises a human IgG4 heavy chain constant domain comprising the amino acid sequence set forth as SEQ ID NO: 5. In certain embodiments, the amino acid sequence of the heavy chain constant domain consists of the sequence set forth as SEQ ID NO: 5.

The amino acid sequences of SEQ ID NOs: 4, 5, and 29-31 are shown in **Table 4**.

Table 4. Heavy Chain Constant Domains

ID	Sequence	SEQ ID NO:
Human IgG1 (UniProt)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPE**LL**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEAL**HN**HYTQKSLSLSPGK	29
Human IgG1 LALA NHance (ARGX-117)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEP KSCDKTHTCPPCPAPE**AA**GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS HEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEAL**KF**HYTQKSLSLSPG	4
Human IgG4 (UniProt)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES KYGPPCP**S**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLS**L**GK	30
Human IgG4 S228P L445P	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES KYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEALHNHYTQKSLSLS**P**GK	31
Human IgG4	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVES	5

S228P NHance L445P	KYGPPCP**P**CPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQED PEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEG NVFSCSVMHEAL**KF**HYTQKSLSLS**P**GK	

In certain embodiments, the monoclonal antibody comprises a full-length monoclonal antibody.

In certain embodiments, the monoclonal antibody consists of a full-length monoclonal antibody.

In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 32. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 32. In certain embodiments, provided herein are monoclonal antibodies comprising a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a light chain with at 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 32, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 32, and a light chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7.

In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 6. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 6. In certain embodiments, provided herein are monoclonal antibodies comprising a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the

amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a light chain with at 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 6, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 6, and a light chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7.

In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 33. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 33. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 33, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 33, and a light chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7.

In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 34. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 34. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 34, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid

sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 34, and a light chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7.

In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 8. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 8. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 8, and a light chain with at least 90%, at least 95%, at least 97%, at least 98%, or at least 99% sequence identity to the amino acid sequence shown as SEQ ID NO: 7. In certain embodiments, provided herein are monoclonal antibodies comprising a heavy chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 8, and a light chain with 100% sequence identity to the amino acid sequence shown as SEQ ID NO: 7.

The amino acid sequences of SEQ ID NOs: 6-8 and 32-34 are shown in **Table 5**.

Table 5. Heavy Chains and Light Chains

ID	Sequence	SEQ ID NO:
Human IgG1 (UniProt)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	32
Human IgG1 LALA NHance (ARGX-117)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYI CNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKD TLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALKFHYTQKSLSLSPG	6

Human IgG4 (UniProt)	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK	33
Human IgG4 S228P L445P	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSPGK	34
Human IgG4 S228P NHance L445P	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDYNMDWVRQATGQGLEWIGD INPNYESTGYNQKFKGRATMTVDKSISTAYMELSSLRSEDTAVYYCARED DHDAFAYWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYT CNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLP PSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALKFHYTQKSLSLSPGK	8
Light Chain (ARGX-117)	DNVLTQSPDSLAVSLGERATISCRASKSVRTSGYNYMHWYQQKPGQPPKL LIYLASNLKSGVPDRFSGSGSGTDFTLTISSLQAEDAATYYCQHSRELPY TFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEV THQGLSSPVTKSFNRGEC	7

For embodiments wherein the heavy and/or light chains of the antibodies are defined by a particular percentage sequence identity to a reference sequence, the heavy chain and/or light chain may retain identical CDR sequences to those present in the reference sequence such that the variation is present only outside the CDR regions.

Unless otherwise stated in the present application, % sequence identity between two amino acid sequences may be determined by comparing these two sequences aligned in an optimum manner and in which the amino acid sequence to be compared can comprise additions or deletions with respect to the reference sequence for an optimum alignment between these two sequences. The percentage of identity is calculated by determining the number of identical positions for which the amino acid residue is identical between the two sequences, by dividing this number of identical positions by the total number of positions in the comparison window and by multiplying the result obtained by 100 in order to obtain the percentage of identity between these two sequences. For example, it is possible to use the

BLAST program, "BLAST 2 sequences" (Tatusova et al, "Blast 2 sequences - a new tool for comparing protein and nucleotide sequences", *FEMS Microbiol Lett.* 174:247-250), the parameters used being those given by default (in particular for the parameters "open gap penalty": 5, and "extension gap penalty": 2; the matrix chosen being, for example, the matrix "BLOSUM 62" proposed by the program), the percentage of identity between the two sequences to be compared being calculated directly by the program.

In non-limiting embodiments, the antibodies of the present invention may comprise CH1 domains and/or CL domains (from the heavy chain and light chain, respectively), the amino acid sequence of which is fully or substantially human. Where the antibody or antigen-binding fragment of the invention is an antibody intended for human therapeutic use, it is typical for the entire constant region of the antibody, or at least a part thereof, to have fully or substantially human amino acid sequence. Therefore, one or more or any combination of the CL domain, CH1 domain, hinge region, CH2 domain, CH3 domain and CH4 domain (if present) may be fully or substantially human with respect to its amino acid sequence.

Advantageously, the CL domain, CH1 domain, hinge region, CH2 domain, CH3 domain and CH4 domain (if present) may all have fully or substantially human amino acid sequence. In the context of the constant region of a humanized or chimeric antibody, or an antibody fragment, the term "substantially human" refers to an amino acid sequence identity of at least 90%, or at least 92%, or at least 95%, or at least 97%, or at least 99% with a human constant region. The term "human amino acid sequence" in this context refers to an amino acid sequence which is encoded by a human immunoglobulin gene, which includes germline, rearranged and somatically mutated genes. The invention also contemplates polypeptides comprising constant domains of "human" sequence which have been altered, by one or more amino acid additions, deletions or substitutions with respect to the human sequence, excepting those embodiments where the presence of a "fully human" hinge region is expressly required.

The presence of a "fully human" hinge region in the C2-binding antibodies of the invention may be beneficial both to minimize immunogenicity and to optimize stability of the antibody.

The C2 binding antibodies may be modified within the Fc region to increase binding affinity for the neonatal Fc receptor FcRn. The increased binding affinity may be measurable at acidic pH (for example from about approximately pH 5.5 to approximately

pH 6.0). The increased binding affinity may also be measurable at neutral pH (for example from approximately pH 6.9 to approximately pH 7.4). In this embodiment, by "increased binding affinity" is meant increased binding affinity to FcRn relative to binding affinity of unmodified Fc region. Typically the unmodified Fc region will possess the wild-type amino acid sequence of human IgG1, IgG2, IgG3 or IgG4. In such embodiments, the increased binding affinity to FcRn of the antibody molecule having the modified Fc region will be measured relative to the binding affinity of wild-type IgG1, IgG2, IgG3 or IgG4 for FcRn.

The C2 binding antibodies may be modified within the Fc region to increase binding affinity for the human neonatal Fc receptor FcRn. The increased binding affinity may be measurable at acidic pH (for example from about approximately pH 5.5 to approximately pH 6.0). The increased binding affinity may also be measurable at neutral pH (for example from approximately pH 6.9 to approximately pH 7.4). In this embodiment, by "increased binding affinity" is meant increased binding affinity to human FcRn relative to binding affinity of unmodified Fc region. Typically the unmodified Fc region will possess the wild-type amino acid sequence of human IgG1, IgG2, IgG3 or IgG4. In such embodiments, the increased binding affinity to human FcRn of the antibody molecule having the modified Fc region will be measured relative to the binding affinity of wild-type IgG1, IgG2, IgG3 or IgG4 for human FcRn.

Pharmaceutical Compositions

An aspect of the invention is a pharmaceutical composition comprising a monoclonal antibody or antigen-binding fragment thereof that specifically binds to human complement factor C2, and a pharmaceutically acceptable carrier, wherein said monoclonal antibody or fragment thereof comprises:

a VH domain comprising the amino acid sequence set forth in SEQ ID NO: 3; and

a VL domain comprising the amino acid sequence set forth in SEQ ID NO: 2.

A pharmaceutical composition of the invention may be formulated with pharmaceutically acceptable carriers or diluents as well as any other known adjuvants and excipients in accordance with conventional techniques such as those disclosed in (Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, Pa., 1995).

The term "pharmaceutically acceptable carrier" relates to carriers or excipients, which are inherently non-toxic. Examples of such excipients are, but are not limited to, saline, Ringer's solution, dextrose solution and Hanks' solution. Non-aqueous excipients such as fixed oils and ethyl oleate may also be used.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The pharmaceutical compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol, sorbic acid, and the like. It may also be desirable to include isotonicity agents, such as sugars, polyalcohols such as mannitol, sorbitol, glycerol or sodium chloride in the compositions. Pharmaceutically-acceptable antioxidants may also be included, for example (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Sterile injectable solutions can be prepared by incorporating the monoclonal antibody in the required amount in an appropriate solvent with one or a combination of ingredients, e.g., as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients, e.g., from those enumerated above. In the case of sterile powders for the preparation of sterile

injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Any reference in the description to methods or in vivo diagnosis refer to the compounds, pharmaceutical compositions and medicaments of the present invention for use in methods of treatment of the human or animal body by therapy or for in vivo diagnosis.

The pharmaceutical composition is preferably administered parenterally, preferably by intravenous (i.v.) or subcutaneous (s.c.) injection or infusion.

The phrases "parenteral administration" and "administered parenterally" as used herein mean modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, intravenous, intraperitoneal, subcutaneous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion.

Prolonged absorption of the injectable anti-C2 mAbs or fragments thereof can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The mAbs or fragments thereof can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

The pharmaceutical compositions can be administered with medical devices known in the art.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Actual dosage levels of the mAbs or fragments thereof in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active

ingredient which is effective to achieve the desired therapeutic response for a particular patient without being toxic to the patient.

In one embodiment, the binding molecules, in particular antibodies, according to the invention can be administered by infusion in a weekly dosage of from 10 to 500 mg/m^2, such as of from 200 to 400 mg/m^2. Such administration can be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 1 to 24 hours, such as a period of from 2 to 12 hours. In some embodiments, administration may be performed by one or more bolus injections.

In one embodiment, the binding molecules, in particular antibodies, according to the invention can be administered by infusion in a weekly dosage of from 1 to 50 mg per kg body weight (mg/kg), such as from 5 to 25 mg/kg. Such administration can be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 1 to 24 hours, such as a period of from 2 to 12 hours. In some embodiments, administration may be performed by one or more bolus injections.

In yet another embodiment, the mAbs or antigen-binding fragments thereof or any other binding molecules disclosed in this invention, can be administered as maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

Nucleic Acid Molecules and Vectors

An aspect of the invention is a nucleic acid molecule or plurality of nucleic acid molecules encoding the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a single nucleic acid molecule encodes both the VH and the VL domains of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a single nucleic acid molecule encodes both the heavy chain (HC) and the light chain (LC) of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a first nucleic acid molecule encodes the VH domain, and a second nucleic acid molecule encodes the VL domain of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a first nucleic acid molecule encodes the heavy chain (HC), and a second nucleic acid molecule encodes the light chain (LC) of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention.

In certain embodiments, a nucleic acid molecule encoding the VH domain comprises the nucleic acid sequence set forth as SEQ ID NO: 35.

In certain embodiments, a nucleic acid molecule encoding the VL domain comprises the nucleic acid sequence set forth as SEQ ID NO: 36.

In certain embodiments, a nucleic acid molecule encoding the HC comprises the nucleic acid sequence set forth as SEQ ID NO: 37.

In certain embodiments, a nucleic acid molecule encoding the HC comprises the nucleic acid sequence set forth as SEQ ID NO: 38.

In certain embodiments, a nucleic acid molecule encoding the HC comprises the nucleic acid sequence set forth as SEQ ID NO: 39.

In certain embodiments, a nucleic acid molecule encoding the HC comprises the nucleic acid sequence set forth as SEQ ID NO: 40.

In certain embodiments, a nucleic acid molecule encoding the HC comprises the nucleic acid sequence set forth as SEQ ID NO: 41.

In certain embodiments, a nucleic acid molecule encoding the LC domain comprises the nucleic acid sequence set forth as SEQ ID NO: 42.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the VH domain consists of the sequence set forth as SEQ ID NO: 35.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the VL domain consists of the sequence set forth as SEQ ID NO: 36.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the HC consists of the sequence set forth as SEQ ID NO: 37.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the HC consists of the sequence set forth as SEQ ID NO: 38.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the HC consists of the sequence set forth as SEQ ID NO: 39.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the HC consists of the sequence set forth as SEQ ID NO: 40.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the HC consists of the sequence set forth as SEQ ID NO: 41.

In certain embodiments, the nucleic acid sequence of a nucleic acid molecule encoding the LC domain consists of the sequence set forth as SEQ ID NO: 42.

The nucleic acid sequences corresponding to SEQ ID NOs: 35-42 are shown in **Table 6**.

Table 6. Nucleic Acid Sequences of VH, VL, HC, and LC

ID	Sequence	SEQ ID NO:
BRO2-IgG4 VH4.2	gaagtgcagctggtgcagtctggcgccgaagtgaagaaacctggcgcctc cgtgaaggtgtcctgcaaggcttccggctacacctttaccgactacaaca tggactgggtgcgacaggctaccggccagggcctggaatggatcggcgac atcaaccccaactacgagtccaccggctacaaccagaagttcaagggcag agccaccatgaccgtggacaagtccatctccaccgcctacatggaactgt cctccctgcggagcgaggacaccgccgtgtactactgcgccagagaggac gaccacgacgcctttgcttattggggccagggcaccctcgtgaccgtgtc ctct	35
BRO2 VL	gacaacgtgctgacccagtcccctgactccctggctgtgtctctgggcga gagagccaccatctcttgccgggcctctaagtccgtgcggacctccggct acaactacatgcactggtatcagcagaagcccggccagccccccaagctg ctgatctacctggcctccaacctgaagtccggcgtgcccgacagattctc cggctctggctctggcaccgactttaccctgaccatcagctccctgcagg ccgaggatgccgccacctactactgccagcactccagagagctgccctac acctttggccagggcaccaagctggaaatcaag	36
BRO2-hIgG1 HC	gaagttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctc tgtgaaggtgtcctgcaaggcttctggctacacctttaccgactacaaca tggactgggtccgacaggctaccggacagggacttgagtggatcggcgac atcaaccccaactacgagtccaccggctacaaccagaagttcaagggcag agccaccatgaccgtggacaagtccatctccaccgcctacatggaactgt ccagcctgagatctgaggacaccgccgtgtactactgcgccagagaggat gatcacgacgcctttgcttattggggccagggcacactggtcaccgtgtc ctctgccagtacaaaaggtccaagtgtgttccctcttgctccctcatcca agagtaccagtggaggcaccgccgctcttggctgcttggttaaggattat ttcccagagcctgtcactgtttcatggaactccggcgccttgacatctgg tgtgcatacctttccagccgtgctgcagtcaagtggcctctacagcctca gtagcgtggtcactgtgcccagcagctctctcggcacacaaacttatatc tgtaatgtgaatcataagccttcaaataccaaggtggataagaaagtgga accaaaatcatgtgacaagacacacacctgccctccttgtccagcccccg aactgctgggtgggcccagcgtgttcctgtttcctcctaaacccaaagac actctgatgattagtaggaccccagaagtcacttgcgtggtggttgacgt gtcacatgaagatcccgaggtcaagttcaattggtatgttgacggggtcg aagttcacaacgctaaaactaaaccaagagaggaacagtataactctacc taccgggtggtgagtgttctgactgtcctccatcaagactggctgaatgg caaagaatacaagtgtaaggtgagcaacaaagccctgcccgctcctatag agaaaacaatatccaaagccaaaggtcaacctcgcgagccacaggtgtac accctcccaccaagccgcgatgaacttactaagaaccaagtctctcttac ttgcctggttaaggggttctatccatccgacattgcagtcgagtgggagt ctaatggacagcctgagaacaactacaaaaccacccctcctgttctggat tctgacggatctttcttcctttattctaaactcaccgtggataaaagcag gtggcagcagggcaacgtgttcagctgttccgttatgcatgaggccctgc ataaccattatacccagaagtctttgtccctcagtccaggaaag	37
BRO2-hIgG1-	gaagttcagctggttcagtctggcgccgaagtgaagaaacctggcgcctc tgtgaaggtgtcctgcaaggcttctggctacacctttaccgactacaaca tggactgggtccgacaggctaccggacagggacttgagtggatcggcgac	38

LALA-NH HC	atcaaccccaactacgagtccaccggctacaaccagaagttcaagggcag agccaccatgaccgtggacaagtccatctccaccgcctacatggaactgt ccagcctgagatctgaggacaccgccgtgtactactgcgccagagaggat gatcacgacgcctttgcttattggggccagggcacactggtcaccgtgtc ctctgcttctaccaagggacccagcgtgttccctctggctccttccagca agtctacctctggcggaacagctgctctgggctgcctggtcaaggactac tttcctgagcctgtgaccgtgtcttggaactctggcgctctgacatctgg cgtgcacacctttccagctgtgctgcagtcctccggcctgtactctctgt cctctgtcgtgaccgtgccttccagctctctgggaacccagacctacatc tgcaatgtgaaccacaagccttccaacaccaaggtggacaagaaggtgga acccaagtcctgcgacaagacccacacctgtcctccatgtcctgctccag aagctgctggcggcccttccgtgtttctgttccctccaaagcctaaggac accctgatgatctctcggacccctgaagtgacctgcgtggtggtggatgt gtctcacgaggacccagaagtgaagttcaattggtacgtggacggcgtgg aagtgcacaacgccaagaccaagcctagagaggaacagtacaactccacc tacagagtggtgtccgtgctgaccgtgctgcaccaggattggctgaacgg caaagagtacaagtgcaaggtgtccaacaaggccctgcctgctcctatcg aaaagaccatctccaaggccaagggccagcctagggaaccccaggtttac accttgcctccatctcgggacgagctgaccaagaaccaggtgtccctgac ctgtctcgtgaagggcttctacccctccgatatcgccgtggaatgggagt ctaatggccagccagagaacaactacaagacaacccctcctgtgctggac tccgacggctcattctttctgtactccaagctgacagtggataagtcccg gtggcagcagggcaacgtgttctcctgttctgtgatgcacgaggccctga agttccactacacacagaagtctctgtctctgagccccggc	
BRO2-hIgG4 HC	gaagtgcagctggtgcagtctggcgccgaagtgaagaaacctggcgcctc cgtgaaggtgtcctgcaaggcttccggctacacctttaccgactacaaca tggactgggtgcgacaggctaccggccagggcctggaatggatcggcgac atcaaccccaactacgagtccaccggctacaaccagaagttcaagggcag agccaccatgaccgtggacaagtccatctccaccgcctacatggaactgt cctccctgcggagcgaggacaccgccgtgtactactgcgccagagaggac gaccacgacgcctttgcttattggggccagggcaccctcgtgaccgtgtc ctctgcttctaccaagggcccctccgtgttccctctggcccccttgctcca gatccacctccgagtctaccgccgctctgggctgcctcgtgaaggactac ttccccgagcccgtgacagtgtcttggaactctggcgccctgacctccgg cgtgcacacctttccagctgtgctgcagtcctccggcctgtactccctgt cctccgtcgtgactgtgccctccagctctctgggcaccaagacctacacc tgtaacgtggaccacaagccctccaacaccaaggtggacaagcgggtgga atctaagtacggccctccctgccctccttgcccagcccctgaatttctgg gcggacccagcgtgttcctgttcccccccaaagcccaaggacaccctgatg atctcccggacccccgaagtgacctgcgtggtggtggatgtgtcccagga agatcccgaggtgcagttcaattggtacgtggacggcgtggaagtgcaca acgccaagaccaagcctagagaggaacagttcaactccacctaccgggtg gtgtccgtgctgaccgtgctgcaccaggattggctgaacggcaaagagta caagtgcaaggtgtccaacaagggcctgccttccagcatcgaaaagacca tctccaaggccaagggccagccccgggaaccccaggtgtacacactgcct ccaagccaggaagagatgaccaagaaccaggtgtccctgacctgtctcgt gaaaggcttctacccctccgatatcgccgtggaatgggagtccaacggcc agcctgagaacaactacaagaccacccccccctgtgctggactccgacggc tccttcttcctgtactctcgcctgaccgtggataagtcccggtggcagga aggcaacgtgttctcctgctccgtgatgcacgaggccctgcacaaccact atacccagaagtccctgtccctgtctctgggaaag	39
BRO2-hIgG4-S228P-L445P HC	gaagtgcagctggtgcagtctggcgccgaagtgaaaaaacctggcgcctc cgtgaaggtgtcctgcaaggctagcggctacacctttaccgactacaaca tggactgggtccgacaggccacaggacagggactcgagtggatcggcgac atcaaccccaactacgagagcaccggctacaaccagaagttcaagggcag	40

	agccaccatgaccgtggacaagagcatcagcaccgcctacatggaactga gcagcctgagaagcgaggacaccgccgtgtactactgcgccagagaggat gatcacgacgcctttgcctattggggccagggcacactggtcaccgttag ctctgctagcaccaagggcccatcggtcttcccccctggcgccctgctcca ggagcacctccgagagcacagccgccctgggctgcctggtcaaggactac ttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcgg cgtgcacaccttcccggctgtcctacagtcctcaggactctactccctca gcagcgtggtgaccgtgccctccagcagcttgggcacgaagacctacacc tgcaacgtagatcacaagcccagcaacaccaaggtggacaagagagttga gtccaaatatggtcccccatgcccaccatgcccagcacctgagttcctgg ggggaccatcagtcttcctgttcccccaaaacccaaggacactctcatg atctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccagga agaccccgaggtccagttcaactggtacgtggatggcgtggaggtgcata atgccaagacaaagccgcgggaggagcagttcaacagcacgtaccgtgtg gtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagta caagtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaacca tctccaaagccaaagggcagccccgagagccacaggtgtacaccctgccc ccatcccaggaggagatgaccaagaaccaggtcagcctgacctgcctggt caaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggc agccggagaacaactacaagaccacgcctcccgtgctggactccgacggc tccttcttcctctacagcaggctcaccgtggacaagagcaggtggcagga ggggaatgtcttctcatgctccgtgatgcatgaggctctgcacaaccact acacacagaagagcctctccctgtctccgggtaaatgagtcctagctgg	
BRO2-hIgG4-S228P-NH-L445P HC	gaagtgcagctggtgcagtctggcgccgaagtgaaaaaacctggcgcctc cgtgaaggtgtcctgcaaggctagcggctacacctttaccgactacaaca tggactgggtccgacaggccacaggacagggactcgagtggatcggcgac atcaaccccaactacgagagcaccggctacaaccagaagttcaagggcag agccaccatgaccgtggacaagagcatcagcaccgcctacatggaactga gcagcctgagaagcgaggacaccgccgtgtactactgcgccagagaggat gatcacgacgcctttgcctattggggccagggcacactggtcaccgttag ctctgctagcaccaagggcccatcggtcttcccccctggcgccctgctcca ggagcacctccgagagcacagccgccctgggctgcctggtcaaggactac ttccccgaaccggtgacggtgtcgtggaactcaggcgccctgaccagcgg cgtgcacaccttcccggctgtcctacagtcctcaggactctactccctca gcagcgtggtgaccgtgccctccagcagcttgggcacgaagacctacacc tgcaacgtagatcacaagcccagcaacaccaaggtggacaagagagttga gtccaaatatggtcccccatgcccaccatgcccagcacctgagttcctgg ggggaccatcagtcttcctgttcccccaaaacccaaggacactctcatg atctcccggacccctgaggtcacgtgcgtggtggtggacgtgagccagga agaccccgaggtccagttcaactggtacgtggatggcgtggaggtgcata atgccaagacaaagccgcgggaggagcagttcaacagcacgtaccgtgtg gtcagcgtcctcaccgtcctgcaccaggactggctgaacggcaaggagta caagtgcaaggtctccaacaaaggcctcccgtcctccatcgagaaaacca tctccaaagccaaagggcagccccgagagccacaggtgtacaccctgccc ccatcccaggaggagatgaccaagaaccaggtcagcctgacctgcctggt caaaggcttctaccccagcgacatcgccgtggagtgggagagcaatgggc agccggagaacaactacaagaccacgcctcccgtgctggactccgacggc tccttcttcctctacagcaggctcaccgtggacaagagcaggtggcagga ggggaatgtcttctcatgctccgtgatgcatgaggctctgaagttccact acacacagaagagcctctccctgtctccgggtaaa	41
BRO2 LC	gacaacgtgctgacccagtcccctgactccctggctgtgtctctgggcga gagagccaccatctcttgccgggcctctaagtccgtgcggacctccggct acaactacatgcactggtatcagcagaagcccggccagccccccaagctg ctgatctacctggcctccaacctgaagtccggcgtgcccgacagattctc cggctctggctctggcaccgactttaccctgaccatcagctccctgcagg	42

	ccgaggatgccgccacctactactgccagcactccagagagctgccctac acctttggccagggcaccaagctggaaatcaagcggaccgtggccgctcc ctccgtgttcatcttcccaccttccgacgagcagctgaagtctggcacag cctccgtcgtgtgcctgctgaacaacttctacccccgcgaggccaaggtg cagtggaaggtggacaacgccctgcagtccggcaactcccaggaatccgt gaccgagcaggactccaaggacagcacctactccctgtcctccaccctga ccctgtccaaggccgactacgagaagcacaaggtgtacgcctgcgaagtg acccaccagggcctgtctagccccgtgaccaagtctttcaaccggggcga gtgc	

For SEQ ID NOs: 35 and 39, a217g creates N72D mutation

The invention further provides a gene delivery vehicle or vector comprising a nucleic acid molecule according to the invention. The gene delivery vehicle or vector can be a plasmid or other bacterially replicated nucleic acid. Such a gene delivery vehicle or vector can be easily transferred to, for instance, producer cells. The gene delivery vehicle can also be a viral vector. Preferred viral vectors are adenoviral vectors, lentiviral vectors, adeno-associated viral vectors and retroviral vectors.

The invention further provides vectors comprising a nucleic acid molecule or a plurality of nucleic acid molecules in accordance with the invention. In certain embodiments, a single vector comprises a single nucleic acid molecule encoding both the VH and the VL domains of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a single vector comprises a single nucleic acid molecule encoding both the heavy chain (HC) and the light chain (LC) of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention.

In certain embodiments, a first vector comprises a first nucleic acid molecule encoding the VH domain, and a second vector comprises a second nucleic acid molecule encoding the VL domain of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, a first vector comprises a nucleic acid molecule encoding the heavy chain (HC), and a second vector comprises a second nucleic acid molecule encoding the light chain (LC) of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention.

Vectors in accordance with the invention include expression vectors suitable for use in expressing the monoclonal antibody or antigen-binding fragment thereof by a host cell. Host cells can be eukaryotic or prokaryotic.

The invention provides a host cell comprising a nucleic acid molecule or plurality of nucleic acid molecules encoding an antibody or antigen-binding fragment thereof in accordance with the instant invention. Alternatively or in addition, the invention provides a

host cell comprising a vector or plurality of vectors encoding an antibody or antigen-binding fragment thereof in accordance with the instant invention. The nucleic acid molecule or molecules, or similarly the vector or vectors, can be introduced into the host cell using any suitable technique, including, for example and without limitation, transduction, transformation, transfection, and injection. Various forms of these methods are well known in the art, including, e.g., electroporation, calcium phosphate transfection, lipofection, cell squeezing, sonoporation, optical transfection, and gene gun.

In certain embodiments, a host cell is a eukaryotic cell. In certain embodiments, a host cell is a yeast cell. In certain embodiments, a host cell is an insect cell. In certain embodiments, a host cell is a mammalian cell. In certain embodiments, a host cell is a human cell. In certain embodiments, a host cell is a mammalian cell selected from the group consisting of hybridoma cells, Chinese hamster ovary (CHO) cells, NS0 cells, human embryonic kidney (HEK293) cells, and PER.C6™ cells. The invention further contemplates other host cells in addition to those mentioned above. Host cells further include cell lines developed for commercial production of the antibodies and antigen-binding fragments thereof in accordance with the invention.

Cell lines provided with the nucleic acid can produce the binding molecule/antibody in the laboratory or production plant. Alternatively, the nucleic acid is transferred to a cell in the body of an animal in need thereof and the binding molecule/antibody is produced *in vivo* by the transformed cell. The nucleic acid molecule of the invention is typically provided with regulatory sequences to the express the binding molecule in the cell. However, present day homologous recombination techniques have become much more efficient. These techniques involve for instance double stranded break assisted homologous recombination, using site-specific double stranded break inducing nucleases such as TALEN. Such or analogous homologous recombination systems can insert the nucleic acid molecule into a region that provides one or more of the *in cis* required regulatory sequences.

The invention further provides an isolated or recombinant cell, or *in vitro* cell culture cell comprising a nucleic acid molecule or vector according to the invention. The invention further provides an isolated or recombinant cell, or *in vitro* cell culture cell comprising a binding molecule according to the invention. Preferably said cell produces said binding molecule. In certain embodiments, said cell secretes said binding molecule. In a preferred embodiment said cell is a hybridoma cell, a CHO cell, an NS0 cell, a HEK293

cell, or a PER-C6™ cell. In a particularly preferred embodiment said cell is a CHO cell. Further provided is a cell culture comprising a cell according to the invention. Various institutions and companies have developed cell lines for the largescale production of antibodies, for instance for clinical use. Non-limiting examples of such cell lines are CHO cells, NS0 cells or PER.C6™ cells. These cells are also used for other purposes such as the production of proteins. Cell lines developed for industrial scale production of proteins and antibodies are herein further referred to as industrial cell lines. Also described is an industrial cell line comprising a nucleic acid molecule, a binding molecule and/or antibody according to the invention. Also described is a cell line developed for the largescale production of protein and/or antibody comprising a binding molecule or antibody of the invention. Also described is the use of a cell line developed for the largescale production of a binding molecule and/or antibody of the invention.

Methods of Making Antibodies

The invention further provides a method of making a monoclonal antibody or antigen-binding fragment thereof in accordance with the invention, comprising culturing a population of host cells according to the invention under conditions suitable for expression of the monoclonal antibody or antigen-binding fragment thereof, and the method further comprises harvesting said monoclonal antibody or antigen-binding fragment thereof from the culture. Preferably said cell is cultured in a serum-free medium. Preferably said cell is adapted for suspension growth. Further provided is an antibody obtainable by a method for producing an antibody according to the invention. The antibody is preferably purified from the medium of the culture. Preferably said antibody is affinity purified.

Methods of Use

An aspect of the invention is a monoclonal antibody, antigen-binding fragment thereof, or composition comprising same for use in a method of inhibiting activation of classical or lectin pathway in a subject and treating or preventing a disease or condition selected from experimental allergic neuritis, type II collagen-induced arthritis, myasthenia gravis, hemolytic anemia, glomerulonephritis, idiopathic membranous nephropathy, rheumatoid arthritis, systemic lupus erythematosus, immune complex-induced vasculitis, multiple sclerosis, adult respiratory distress syndrome, stroke, xenotransplantation, allotransplantation, burn injuries, sepsis, septic shock, toxicity induced by the in vivo

administration of cytokines or mAbs, antibody-mediated rejection of allografts such as kidney allografts, multiple trauma, ischemia-reperfusion injuries, and myocardial infarction. Although not forming part of the invention, described herein is a method of inhibiting activation of classical or lectin pathway in a subject, comprising administering to a subject in need thereof an effective amount of the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention. In certain embodiments, the subject is a mammal. In certain embodiments, the subject is a mouse, rat, hamster, Guinea pig, rabbit, goat, sheep, pig, cat, dog, horse, or cow. In certain embodiments, a subject is a non-human primate, e.g., a monkey. In certain embodiments, a subject is a human.

The inhibitory effect of the antibody or antigen-binding fragment can be assessed using any suitable method, including, for example, measuring total complement activity, a test of hemolytic activity based on the ability of a serum sample to lyse sheep erythrocytes coated with anti-sheep antibodies. Decreased hemolysis compared to an untreated control sample indicates an inhibitory effect of the antibody or antigen-binding fragment. In an embodiment, the untreated control sample can be a historical sample obtained prior to starting treatment with the antibody or antigen-binding fragment. Generally, a decrease in total complement activity of at least 5% compared to control is indicative of efficacy. In certain embodiments, a decrease in total complement activity of at least 10% compared to control is indicative of efficacy.

Diseases that can be treated or prevented by a monoclonal antibody or antigen-binding fragment thereof in accordance with the invention are diseases such as experimental allergic neuritis, type II collagen-induced arthritis, myasthenia gravis, hemolytic anemia, glomerulonephritis, idiopathic membranous nephropathy, rheumatoid arthritis, systemic lupus erythematosus, immune complex-induced vasculitis, adult respiratory distress syndrome, stroke, xenotransplantation, allotransplantation, multiple sclerosis, burn injuries, extracorporeal dialysis and blood oxygenation, including sepsis and septic shock, toxicity induced by the *in vivo* administration of cytokines or mAbs, antibody-mediated rejection of allografts such as kidney allografts, multiple trauma, ischemia-reperfusion injuries, and myocardial infarction.

Individuals suffering from a disease involving complement-mediated damage or at risk of developing such complement-mediated damage can be treated by administering an effective amount of a monoclonal antibody or antigen-binding fragment thereof in accordance with the invention to an individual in need thereof. Thereby the biologically

active complement-derived peptides are reduced in the individual and the lytic and other damaging effects of complement on cells and tissues is attenuated or prevented. By "effective amount" is meant an amount sufficient to achieve a desired biological response. In an embodiment, by "effective amount" is meant an amount of a monoclonal antibody or antigen-binding fragment thereof in accordance with the invention that is capable of inhibiting complement activation in the individual.

Treatment (prophylactic or therapeutic) will generally consist of administering the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention parenterally together with a pharmaceutical carrier, for example intravenously, subcutaneously, or locally. The administering typically can be accomplished by injection or infusion. The dose and administration regimen of the monoclonal antibody or antigen-binding fragment thereof in accordance with invention will depend on the extent of inhibition of complement activation aimed at. Typically, for monoclonal antibodies of the invention, the amount will be in the range of 2 to 20 mg per kg of body weight. For parenteral administration, the monoclonal antibody or antigen-binding fragment thereof in accordance with the invention will be formulated in an injectable form combined with a pharmaceutically acceptable parenteral vehicle. Such vehicles are well-known in the art and examples include saline, dextrose solution, Ringer's solution and solutions containing small amounts of human serum albumin.

Pharmaceutical compositions typically must be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, micro-emulsion, liposome, or other ordered structure suitable to high drug concentration. Examples of suitable aqueous and non-aqueous carriers which may be employed in the pharmaceutical compositions of the invention include water, ethanol, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, such as olive oil, and injectable organic esters, such as ethyl oleate. Proper fluidity can be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

The pharmaceutical compositions may also contain adjuvants such as preservatives, wetting agents, emulsifying agents and dispersing agents. Prevention of presence of microorganisms may be ensured both by sterilization procedures and by the inclusion of various antibacterial and antifungal agents, for example, paraben, chlorobutanol, phenol,

sorbic acid, and the like. It may also be desirable to include isotonicity agents, such as sugars, polyalcohols such as mannitol, sorbitol, glycerol or sodium chloride in the compositions. Pharmaceutically-acceptable antioxidants may also be included, for example (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Sterile injectable solutions can be prepared by incorporating the mAb or fragments thereof in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

Prolonged absorption of the injectable anti-C2 mAbs or fragments thereof can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin.

The mAbs or antigen-binding fragments thereof can be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for the preparation of such formulations are generally known to those skilled in the art. See, e.g., Sustained and Controlled Release Drug Delivery Systems, J. R. Robinson, ed., Marcel Dekker, Inc., New York, 1978.

The pharmaceutical compositions can be administered with medical devices known in the art.

Dosage regimens are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided

doses may be administered over time, or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation.

Actual dosage levels of the mAbs or fragments thereof in the pharmaceutical compositions of the present invention may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient without being toxic to the patient.

In one embodiment, the monoclonal antibodies according to the invention can be administered by infusion in a weekly dosage of from 10 to 500 mg/m^2, such as of from 200 to 400 mg/m^2. Such administration can be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours.

Monoclonal antibodies or antigen-binding fragments thereof can be administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

The present invention will now be illustrated with reference to the following examples, which set forth particularly advantageous embodiments. However, it should be noted that these embodiments are merely illustrative and are not to be construed as restricting the invention in any way. The invention is limited only by the scope of the appended claims.

EXAMPLES

Example 1: Removal of a Glycosylation Site from an Anti-C2b Monoclonal Antibody

BRO2-glyc-IgG4

U.S. Patent No. 9,944,717 discloses a murine inhibitory anti-C2b monoclonal antibody (mAb). From this lead, four humanized variants, comprising different heavy chain variable domains (VH1, VH2, VH3, or VH4) and kappa light chain variable domains (VK1, VK2, VK3, or VK4), were generated using the Composite Human Antibody technology of Antitope Ltd (Cambridge, UK). Based on *in silico* analysis, the risk of immunogenicity for each of the humanized VH and VK sequences was predicted. As shown in **Table 7**, the lowest risk for immunogenicity, along with the highest percentage of identity to the closest human germline variant, was predicted when VH4 was paired with VK3 or VK4. This observation was based on the lowest number of promiscuous binding peptides to human MHC class II. VH4 was preferred because of its higher percentage of identity against the closest human germline. In addition, based on binding and potency, VH4/VK3 was selected

as the anti-human C2b humanized lead antibody and is referred to herein as BRO2-glyc-IgG4.

Table 7. Risk for immunogenicity ranked 1 (=lowest) to 5 (=highest) (high affinity priority over moderate affinity) and sequence identity to the closest human germline

VH	High Affinity	Moderate Affinity	Ranking	Identity to IGHV1-8*01	VL	High Affinity	Moderate Affinity	Ranking	Identity to IGKV4-1*01
WT	1	2	5	79.3%	WT	6	5	5	80.0%
VH1	0	3	4	86.2%	VK1	3	3	3	92.5%
VH2	0	2	1	90.8%	VK2	3	3	3	95.0%
VH3	0	2	1	93.1%	VK3	3	2	1	96.3%
VH4	0	2	1	95.4%	VK4	3	2	1	97.5%

SDS-PAGE analysis of variants of BRO2-glyc-IgG4 revealed a double band and band shift in the VH3 and VH4 variants. This shift was hypothesized to arise from a potential glycosylation site (motif NXS) at residues 72-74 (Kabat numbering) in framework region 3 (FR3) of VH3 and VH4. Because this potential glycosylation site could result in heterogeneity not only of antibody product expressed from mammalian cell lines, but also of antibody function, the potential glycosylation site was removed. The glycosylation site was removed by site-directed mutagenesis to generate an N72D variant of the VH, referred to herein as either VH3.2 or VH4.2. The N72D mutation removed the altered band pattern observed in VH3 and VH4 (Fig. 1), confirming that the double band and band shift was caused by glycosylation and heterogeneity in the heavy chain.

To further determine whether variant VH4.2, which is the same VH as BRO2-glyc-IgG4 but without the glycosylation site in FR3, demonstrated improved characteristics compared to the heterogeneously glycosylated parent mAb BRO2-glyc-IgG4, thermotolerance of each antibody was determined.

To test thermotolerance, humanized variants were treated with an increasing temperature from 55°C up to 75°C with Thermocycler (Biometra). Residual binding capacity was analyzed on Biacore 3000 on a CM5 Chip directly coated with human C2 purified from serum (3500 RU, Complement Technologies Cat#A112, lot#20). Data were analyzed using the BIAevaluation software. The slope of specific binding of each variant was determined with the BIAevaluation software, general fit from the linear phase of the sensorgram (started at 5 seconds after the start of injection and stopped after 11 seconds). Then percentage of activity was calculated, using the mean of the slope obtained for the 59°C, 56.9°C, 55°C and 4°C temperatures as 100% activity. Finally, the percentage of activity was plotted in GraphPad Prism (Log (agonist) vs response, variable slope (4 parameters)). The temperature where the antibody lost 50% of its binding capacity (TM50) is shown in **Table 8** below.

BRO2-IgG4

Both variants without the glycosylation site present in BRO2-glyc-IgG4 demonstrated improved thermotolerance (**Table 8**). BRO2-glyc-IgG4 exhibited a TM50 of 64.0 °C. VH4.2/VK3 (also referred to herein as BRO2-IgG4) exhibited a TM50 of 65.0 or 65.1 °C in two independent experiments. VH4.2/VK4 exhibited a TM50 of 65.2 or 65.4 °C in two independent experiments.

Table 8. Percent Identity to closest human germline sequences and thermotolerance of Anti-C2b Monoclonal Antibodies

	BRO2-glyc-IgG4	VH4.2/VK3 (BRO2-IgG4)	VH4.2/VK4
% Identity to closest human germline sequences	95.8	95.3	95.9
% Homology to closest human germline sequences	97.0	97.0	97.6
Thermotolerance (TM50, °C)	64.0	65.0; 65.1	65.2; 65.4

Example 2: Preparation of Non-glycosylated IgG4 and Non-glycosylated IgG1 Variants

BRO2-IgG4-NH

Antibodies with pH-dependent antigen binding dissociate bound antigen in acidic endosomes after internalization into cells. Consequently, released antigen is trafficked to the lysosome and degraded, whereas the dissociated antibody, free of antigen, is recycled back to plasma by FcRn. The recycled free antibody can bind to another target antigen. By repeating this cycle, a pH-dependent antigen-binding antibody can bind to the target antigen more than once and therefore improve the neutralizing capacity of the antibody. This process can further be improved when an antibody is equipped with NHance® (NH) technology (argenx, Belgium) that enhances the binding of the antibody to FcRn at acidic endosomal pH (pH 6.0) but not at neutral pH (pH 7.4). Therefore, amino acids in the Fc region of BRO2-IgG4 were mutated to alter pH-dependent binding to FcRn (H433K, N434F). The resulting antibody is referred to herein as BRO2-IgG4-NH.

BRO2-IgG1-NH and

BRO2-IgG1-LALA-NH (ARGX-117)

The effect of immunoglobulin subclass on efficacy was also examined. A further NHance® variant was prepared in a human IgG1 background (BRO2-IgG1-NH). Antibody effector functions can be further diminished by mutations in the Fc region that alter binding of the antibody to Fcγ receptors. Therefore, amino acid substitutions L234A and L235A ("LALA") were incorporated into BRO2-IgG1-NH to yield BRO2-IgG1-LALA-NH, also referred to herein as ARGX-117.

His1-IgG1-LALA-NH

To determine if pH dependency of BRO2-IgG1-LALA-NH could be improved to extend its pharmacokinetic and pharmacodynamic (PK/PD) effects *in vivo*, an amino acid in

the VK of the BRO2-IgG1-LALA-NH antibody was mutated to histidine (G29H, mutant VK referred to herein as Vk3m3). The resulting antibody is referred to herein as His1-IgG1-LALA-NH.

His1-IgG4

Similarly, to determine if pH dependency of BRO2-IgG4 could be improved to extend its PK/PD effects *in vivo*, an amino acid in the VK of the BRO2-IgG4 antibody was mutated to histidine (G29H, mutant VK referred to herein as Vk3m3). The resulting antibody (VH4.2/Vk3m3) is referred to herein as His1-IgG4.

His1-IgG4-NH

To examine the effect of recycling on antibody efficacy, the NHance® mutations were incorporated into the His1-IgG4 (VH4.2/Vk3m3) antibody. The resulting antibody is referred to herein as His1-IgG4-NH.

His2-IgG4-NH

To determine if pH dependency of BRO2-IgG4-NH could be improved to extend its PK/PD effects *in vivo*, an amino acid of the VH4 of the BRO2-IgG4-NH antibody was mutated to histidine (K26H, VH mutant referred to herein as VH4.2m12). Additionally, the VK3 light chain of the BRO2-IgG4-NH antibody was replaced with the VK4 light chain mentioned above, and a second amino acid was mutated to histidine (G29H, VK4 mutant referred to herein as VK4m3). The resulting antibody (VH4.2m12/VK4m3) is referred to herein as His2-IgG4-NH.

Example 3: Efficacy Improvements in Non-glycosylated BRO2 Variants

Total Pharmacokinetics (PK)

Cynomolgus monkeys (n = 2, 1 male and 1 female per group) were randomly assigned into separate treatment groups in accordance with **Table 9** below.

Table 9. Treatment Group Assignments

Group	Antibody	Animal No.
1	BRO2 glyc-IgG4	1 2
2	Negative Control	3 4
3	BRO2-IgG4	5 6
4	BRO2-IgG4-NH	7 8
5	BRO2-IgG1-LALA-NH	9 10
6	His1-IgG4	11 12
7	His1-IgG4-NH	13 14
8	His1-IgG1-LALA-NH	15 16
9	His2-IgG4-NH	17 18

A serum sample was obtained from each monkey one day prior to receiving test antibody (day -1, or "PRE"). Then on day 1 (d1), each monkey received a single intravenous injection of 5 mg/kg test antibody in accordance with **Table 9**. Serum samples were then obtained from each monkey serially over up to 60 days (to d60).

For PK of total antibody (total PK), a microtiter plate was coated overnight at 4°C with 100 µL goat anti-human IgG (Bethyl; A80-319A) at 5 µg/mL. Plates were washed 3 times with at least 200 µL PBS-0.05% Tween20 and subsequently blocked with 200 µL PBS-2% BSA for 2 hours at room temperature (RT). After washing the plates 3 times with at least 200 µL PBS-0.05% Tween20, serum samples, standard and QC samples (prepared in pooled naïve cynomolgus monkey serum) were applied in duplicate at 100-fold dilution or more and diluted in 100 µL PBS-0.2% BSA-1% pooled naïve cynomolgus monkey serum. For each antibody, its own frozen standards and QC samples were applied in duplicate (the same batch of antibody was used as the batch that was injected in the monkeys). The negative control antibody is an antibody that binds a non-C2 complement component. Incubation was done at RT for 2 hours whilst shaking the plate. After washing the plates 5 times with at least 200 µL PBS-0.05% Tween20, 100 µL horseradish peroxidase (HRP)-labeled mouse anti-human IgG kappa (Southern Biotech, 9230-05) was diluted 260,000-fold in PBS 0.2% BSA and applied to the wells for 1 hour at RT. The

plates were washed 5 times with at least 200 µL PBS-0.05% Tween20 and staining was done with 100 µL 3,3',5,5'-tetramethylbenzidine (TMB) and stopped after 10 minutes with 100 µL 0.5 M H_2SO_4 (CHEM LAB, Cat#CL05-2615-1000). The OD was measured at 450 nm and GraphPad Prism was used to back calculate the concentration of samples (each using its own standard).

Results are shown in **Table 10** and a comparison of glycosylated BRO2-glyc-IgG4 with non-glycosylated BRO2-IgG4 is shown in Fig. 2. In the total PK assay, concentrations of non-glycosylated BRO2-IgG4 were generally greater than those of glycosylated BRO2-glyc-IgG4. This improvement in total PK was completely unexpected and represents an important further advantage of the non-glycosylated antibody.

Table 10. Total PK

	Total PK (µg/mL)							
	BRO2-glyc-IgG4				BRO2-IgG4			
	Monkey 1	Monkey 2	average M1&M2	Std Dev	Monkey 5	Monkey 6	average M5&M6	Std Dev
15 min	107.1	99.9	103.4	5.3	167.0	166.7	162.5	5.6
1 h	103.9	99.9	102.2	1.7	172.9	158.4	168.1	13.3
2 h	95.4	89.0	94.8	2.8	151.1	150.9	147.6	3.8
4 h	91.4	92.2	90.0	2.5	134.6	132.3	132.7	1.2
6 h	86.5	86.0	85.4	1.6	134.3	128.9	133.6	5.5
24 h	56.1	55.5	58.3	1.2	99.6	97.6	85.9	1.9
Day 2	47.9	47.2	47.3	0.6	77.6	78.3	68.7	9.3
Day 4	34.8	39.8	34.1	2.7	58.4	55.8	60.8	4.8
Day 7	26.6	25.1	26.0	0.6	41.5	33.6	34.9	7.6
Day 11	16.3	14.1	16.2	1.3	28.1	20.9	24.5	5.1
Day 15	9.8	8.1	9.3	0.9	18.1	11.2	13.0	5.1
Day 19	6.7	5.1	6.5	1.1	12.8	6.5	8.3	2.5
Day 23	4.4	3.2	4.4	0.8	9.8	4.3	7.1	3.9
Day 27	3.2	1.9	3.1	0.7	6.5	2.2	3.9	3.0
Day 31	2,2	1,5	2.3	0.4	4,7	1,0	2.9	2.6

Free C2

Cynomolgus monkeys (n = 2, 1 male and 1 female per group) received a single intravenous injection of 5 mg/kg test antibody, as described above.

In this assay a microtiter plate was coated overnight at 4°C with 100 µL 2.5 µg/mL mouse anti-human C2 monoclonal antibody mAb32 (anti-C2 #32 m-IgG @ 3.31 mg/mL, 0.2 µm PBS, LC-12/05-166, 12-apr-13). This antibody binds to a different epitope on C2 than BRO2. Plates were washed 3 times with at least 200 µL PBS-0.05% Tween20 and subsequently blocked with 200 µL PBS- 2% BSA (pH 7.4) for 2 hours at RT. In the meantime, samples, frozen standard (specific for each antibody, prepared in pooled naïve cynomolgus monkey serum) and frozen QC samples (prepared in pooled naïve cynomolgus monkey serum) were thawed and diluted 6.7-fold in 80 µL PBS-0.2%BSA. 40 µL biotinylated anti-C2 VH4/VK3 was added at 0.6 µg/mL. Each sample was made in duplicate. 100 µL of the mixture was transferred immediately to the washed coated plate after addition of the biotinylated antibody. The plate was incubated for 2 hours at RT, washed 5 times with at least 200 µL PBS-0.02%Tween20, and 100 µL strep-HRP (Jackson, 016-030-084) was added at 300,000-fold dilution in PBS-0.2%BSA. After 1 hour incubation at RT, the plates were washed 5 times with at least 200 µL PBS-0.05%Tween20 and staining was done with 100 µL TMB (Calbiochem, CL07) and stopped after 10 minutes with 100 µL 0.5 M H_2SO_4 (CHEM LAB, Cat#CL05-2615-1000). The OD was measured at 450 nm and used to determine C2 levels.

Sera from the following monkeys were first tested together using the free C2 assay performed on different days: monkeys 1 and 2; monkeys 3 and 4; monkeys 5 and 6; monkeys 7, 8, 9, and 10; monkeys 11, 12, 15, and 16; and monkeys 13, 14, 17, and 18.

The levels of free C2 for all monkeys are shown in Figs. 3A-3I and in **Table 11**.

As expected, for monkeys 3 and 4 there was no decline in free C2, as these monkeys were dosed with a negative control antibody. For all monkeys treated with the BRO2 variants, free C2 levels were very low until after day 2.

For the monkeys receiving BRO2-glyc-IgG4 (monkeys 1 and 2) and BRO2-IgG4 (monkeys 5 and 6), C2 levels went back up beginning at day 4 and were back to baseline levels by day 31. Monkeys 5 and 6, treated with non-glycosylated antibody, consistently displayed lower free C2 levels than those treated with BRO2-glyc-IgG4 (Fig. 3C, **Table 11**).

For all other monkeys, excluding those with anti-drug antibodies (ADA, marked by a * in Figs. 3D-3I), C2 levels increased much more slowly, and C2 levels did not return to baseline even by day 31.

Fig. 4 shows a blow up (log scale) of the free C2 levels (OD 450 nm) for the average of the 2 monkeys of each group. Free C2 levels were lower for BRO2 variants than for His1 variants.

Monkey 10, injected with BRO2-IgG1-LALA-NH (ARGX-117), had the lowest levels of C2 at all time points tested. Comparison of free C2 levels from monkeys 5 and 6, 9 and 10, and 15 and 16 out to 60 days can be seen in Fig. 5. Monkey 10 also had the best total PK (see above). The raw data is shown in **Table 11**, and average data comparing the glycosylated and non-glycosylated variants is shown in **Table 12**.

Table 11. Free C2 (OD450nm) for all antibodies

Time Point	M 1	M 2	M 3	M 4	M 5	M 6	M 7	M 8	M 9	M 10	M 11	M 12	M 13	M 14	M 15	M 16	M 17	M 18
Day -5	0.773	0.770	0.797	0.930	0.696	0.705	0.439	0.532	0.602	0.626	0.656	0.643	0.824	0.805	0.756	0.755	0.789	0.935
Day 0	0.707	0.716	0.813	0.907	0.740	0.657	0.507	0.531	0.578	0.604	0.620	0.687	0.847	0.792	0.772	0.750	0.834	1.043
15 m	0.031	0.028	0.879	0.979	0.016	0.018	0.016	0.014	0.015	0.013	0.029	0.033	0.032	0.038	0.033	0.028	0.050	0.055
1 h	0.033	0.028	0.914	1.055	0.017	0.021	0.014	0.013	0.014	0.013	0.030	0.033	0.035	0.037	0.034	0.031	0.050	0.062
2 h	0.034	0.030	0.874	0.997	0.018	0.020	0.016	0.014	0.015	0.015	0.031	0.031	0.034	0.043	0.034	0.030	0.052	0.063
4 h	0.033	0.027	0.887	1.000	0.017	0.020	0.017	0.015	0.016	0.016	0.037	0.034	0.036	0.044	0.037	0.032	0.054	0.062
6h	0.034	0.030	0.958	1.035	0.015	0.019	0.015	0.014	0.015	0.017	0.032	0.034	0.035	0.043	0.034	0.034	0.052	0.071
Day 1	0.046	0.045	0.917	0.923	0.021	0.025	0.019	0.018	0.020	0.020	0.041	0.038	0.046	0.054	0.048	0.044	0.064	0.084
Day 2	0.060	0.061	0.872	0.920	0.027	0.030	0.021	0.018	0.023	0.022	0.075	0.040	0.050	0.052	0.053	0.043	0.068	0.090
Day 4	0.125	0.102	0.833	0.886	0.037	0.048	0.026	0.021	0.035	0.024	0.088	0.056	0.063	0.064	0.056	0.055	0.080	0.096
Day 7	0.174	0.169	0.853	0.899	0.072	0.110	0.033	0.025	0.050	0.028	0.119	0.085	0.075	0.075	0.070	0.071	0.080	0.099
Day 11	0.257	0.265	0.862	0.847	0.127	0.193	0.050	0.033	0.092	0.033	0.172	0.105	0.088	0.088	0.090	0.087	0.102	0.134
Day 15	0.364	0.375	0.840	0.834	0.177	0.290	0.065	0.043	0.138	0.043	0.315	0.138	0.086	0.096	0.094	0.109	0.133	0.147
Day 19	0.418	0.471	0.807	0.864	0.256	0.406	0.083	0.031	0.194	0.051	0.289	0.157	0.157	0.106	0.113	0.127	0.143	0.167
Day 23	0.517	0.562	0.820	0.897	0.327	0.469	0.100	0.059	0.255	0.062	0.351	0.225	0.339	0.133	0.126	0.148	0.176	0.192
Day 27	0.597	0.633	0.818	0.921	0.378	0.537	0.124	0.146	0.292	0.071	0.418	0.230	0.492	0.255	0.140	0.170	0.199	0.231
Day 31	0.633	0.663	0.841	0.934	0.431	0.599	0.153	0.125	0.364	0.098	0.511	0.280	0.605	0.522	0.163	0.205	0.238	0.244

Table 12. Average Free C2 of Glycosylated and Non-Glycosylated Antibodies

	Free C2 (OD 450nm)							
	BRO2-glyc-IgG4				BRO2-IgG4			
	Monkey 1	Monkey 2	average M1&M2	Standard Deviation	Monkey 5	Monkey 6	average M5&M6	Standard Deviation
Day -5	0.773	0.77	0.772	0.002	0.696	0.705	0.701	0.006
Day 0	0.707	0.716	0.712	0.006	0.74	0.657	0.699	0.059
15 min	0.031	0.028	0.030	0.002	0.016	0.018	0.017	0.001
1 h	0.033	0.028	0.031	0.004	0.017	0.021	0.019	0.003
2 h	0.034	0.03	0.032	0.003	0.018	0.02	0.019	0.001
4 h	0.033	0.027	0.030	0.004	0.017	0.02	0.019	0.002
6 h	0.034	0.03	0.032	0.003	0.015	0.019	0.017	0.003
Day 1	0.046	0.045	0.046	0.001	0.021	0.025	0.023	0.003
Day 2	0.06	0.061	0.061	0.001	0.027	0.03	0.029	0.002
Day 4	0.125	0.102	0.114	0.016	0.037	0.048	0.043	0.008
Day 7	0.174	0.169	0.172	0.004	0.072	0.11	0.091	0.027
Day 11	0.257	0.265	0.261	0.006	0.127	0.193	0.160	0.047
Day 15	0.364	0.375	0.370	0.008	0.177	0.29	0.234	0.080
Day 19	0.418	0.471	0.445	0.037	0.256	0.406	0.331	0.106
Day 23	0.517	0.562	0.540	0.032	0.327	0.469	0.398	0.100

As these assays for the different monkeys just described were run on different days, the analysis was repeated for a select number of time points (pre, 4 hours, days 1, 2, 4, 11, and 27) where sera from all monkeys were put on a single plate (Figs. 6A-6D). The pre-samples were also tested with and without addition of excess BRO2 (500 µg/mL).

The ODs of the pre-samples were comparable for all monkeys, indicating that free C2 levels in the different monkeys were comparable (Fig. 6A). When the pre-samples were pre-incubated with 500 µg/mL BRO2, all signals dropped to an OD of 0.013-0.015 (Figs. 6A and 6B). Such low OD values were not obtained for any of the PK samples, indicating that at no time point was free C2 completely depleted. The lowest levels were obtained at 4 hours, and they were the lowest (OD between 0.02 and 0.03) for the BRO2 variants (monkeys 5, 6, 7, 8, 9, and 10, Fig. 6C). Interpretation of the results at day 11 and day 27 was hampered by ADA (anti-drug antibodies) that was observed in several of the monkeys (Fig. 6D).

Immunogenicity

Cynomolgus monkeys (n = 2, 1 male and 1 female per group) received a single intravenous injection of 5 mg/kg test antibody, as described above. Serum samples obtained from all monkeys were tested for ADA (anti-drug antibodies) from baseline (pre-exposure)

until day 31 (Figs. 7A-7P), and serum samples obtained from monkeys 5 and 6, 9 and 10, and 15 and 16 were further tested until day 59 (Figs. 8A-8F).

Immunogenicity was determined by coating a microtiter plate with 100 µL of 1 µg/mL of the respective antibody overnight at 4°C. Plates were washed 3 times with at least 200 µL PBS-0.05% Tween20 and subsequently blocked with 200 µL PBS-1% casein for 2 hours at RT. After washing the plates 3 times with at least 200 µL PBS-0.05% Tween20, serum samples were diluted 20-fold or more in 100 µL PBS-0.1% casein and incubated in the coated wells for 2 hours at room temperature (RT). After washing the plates 5 times with at least 200 µL PBS-0.05% Tween20, 100 µL anti-monkey IgG-HRP (Southern Biotech #4700-05) was added to the wells at a 8000-fold dilution for 1 hour at RT. The plates were washed 5 times with at least 200 µL PBS-0.05% Tween20 and staining was done with 100 µL TMB and stopped after 10 minutes with 100 µL 0.5 M H_2SO_4 (CHEM LAB, Cat#CL05-2615-1000). The OD was measured at 450 nm. Representative results are shown in Figs. 7A-7P.

A clear ADA response was observed for monkeys 8 (BRO2-IgG4-NH), 11 (His1-IgG4), 13 and 14 (His1-IgG4-NH), and 16 (His1-IgG1-LALA-NH) (Figs. 7F, 7I, 7K and 7L, and 7N, respectively). Indeed, the signal obtained in ELISA after injection of the antibody as compared to the baseline ("PRE") signal (before injection of the antibody) was increased at least 2-fold.

For monkeys 11, 13, and 16 (Figs. 7I, 7K, and 7N, respectively), ADA was observed as of day 11; for monkey 8 (Fig. 7F), as of day 15; and for monkey 14 (Fig. 7L), as of day 19.

For monkey 9 (BRO2-IgG1-LALA-NH) (Fig. 7G), an increase in signal was observed for all samples post injection of the antibody, but the signal in the baseline sample was already high and the increase over time was low (about 1.5-fold).

For monkey 5 (Fig. 7C) an unusually high signal was observed in the baseline sample before injection of the antibody. This signal was also higher than the signals of the later timepoints. This may be explained by the interference of the antibody (present in the serum) with the assay. It was therefore not possible to determine if there was an ADA response in this monkey.

Example 4: Isoelectric Point (pI)

Igawa et al. (*Protein Eng Des Sel* 2010, 23(5):385-392), studying VH mutants of certain IgG1 monoclonal antibodies, reported a strong positive correlation between isoelectric point (pI) and monoclonal antibody clearance and a negative correlation between pI and

monoclonal antibody half-life. In this example, the pI of various forms of anti-human C2b were determined. Results are shown in **Table 13**.

Table 13. Apparent pI of Anti-human C2b Monoclonal Antibodies

	Peak	VH4.2-IgG4-IAP2VK3	VH4.2-IgG4-HN-IAP2VK3	VH4.2-IgG1-LALA-HN-IAP2VK3 (ARGX-117)
Calculated Apparent pI n = 3	Acidic 3	7.02	7.14	
	Acidic 2	7.10	7.24	
	Acidic 1	7.16	7.32	8.29
	Main Peak	7.20	7.35	8.43
	Basic 1	7.30	7.45	8.57
	Basic 2	7.42	7.58	

All three antibodies tested are without glycosylation in VH. As shown in Table 13, the pI of ARGX-117 was found to be significantly greater than the pI of closely related IgG4 antibodies. The observed pI of ARGX-117 is expected to be manifested as enhanced potential for so-called antigen sweeping.

Example 5: Domain Mapping by Western Blotting and Surface Plasmon Resonance (SPR) Analysis

Binding characteristics of ARGX-117 were assessed by Western blotting and by Surface Plasmon Resonance (SPR) analysis, as depicted in Figure 1. Western blotting results revealed that ARGX-117 binds to C2 and C2b, as depicted in **Figure 9A**. The binding characteristics of ARGX-117 were further studied by SPR, using the Biacore 300, by coating C2 (SEQ ID NO: 21) on the solid phase with different concentrations of Fabs of ARGX-117 used as eluate, as depicted in **Figure 9B**. Affinities were calculated assuming 1:1 binding between the Fab and C2 and yielded a Kd of about 0.3 nM. In order to study the mechanism of action by ARGX-117, SPR analysis was performed, mimicking the formation of C3 convertase (C4bC2a) with biotinylated C4 immobilized to streptavidin-coated chips, as depicted in **Figure 9C**. When C2 was added in flowing buffer, alone or preincubated with the control mAb, C2 binding was observed on the chip. Pre-incubation with anti-C2 clone 63 (i.e., anti-C2-63) resulted in higher signal, presumably because this mAb form complexed to C2 and C2:mAb complexes bind together resulting in higher molecular mass and higher SPR signal. When C2 was pre-incubated with ARGX-117, binding of C2 to C4b was greatly reduced. The initial interaction of C2 to C4b is thought to be initiated by the C2b domain

(SEQ ID NO: 44). Thereafter the large C2a domain (SEQ ID NO: 43) takes over and this interaction is crucial in the formation of the C3 convertase complex. The results from this experiment suggest that ARGX-117 inhibits C2 by inhibiting binding to C4b.

To further understand the mechanism of action of C2 inhibition by ARGX-117, C2 was first allowed to bind to C4b immobilized on streptavidin chips, and after stabilization by flowing buffer only, samples were flown, as depicted in **Figure 9D**. Running buffer or control human IgG4 mAb targeting an irrelevant soluble antigen (i.e., anti-Factor XI (anti-FXI)) resulted in some signal decrease, which normalized after injection ceased. Injection of anti-C2-63 resulted in increased signal, suggesting that this mAb is able to bind to C3 convertase (C4bC2a). This is in line with the predicted binding model of C2 to C4b, which suggests that after binding on C2, the C2a domain is still largely available. Interestingly, ARGX-117 demonstrated a strong binding to C3 convertase that was followed by a rapid dissociation. These results suggest that ARGX-117 is able to bind C2, but that this binding is very unstable, likely affecting C2 in a way that facilitates activation. These results also suggest that ARGX-117 would be released together with C2b from the C2 molecule.

Example 6: Domain Mapping Using Domain Swap Mutants of C2 and Factor B

In order to map the epitope of anti-C2-5F2.4, advantage was taken of the fact that anti-C2-5F2.4 does not cross-react with Factor B (FB; SEQ ID NO: 50) and that C2 and FB are highly homologous proteins that have similar domain structure. Both proteins comprise a small fragment, and a large fragment. The small fragment in complement C2 is called C2b (SEQ ID NO:44), and the small fragment in Factor B is called FBa (SEQ ID NO: 51). The small fragment in each comprises three Sushi domains (CCP domains). The large fragment in each comprisesa von Willebrand Factor type A domain (VWFA) and a Peptidase S1 domain on, as shown in **Figure 10**. Domain swap mutants included a C-terminal FLAG tag.

To generate the various swap mutants, DNA constructs for C2, FB, and the ten domain swap mutants were obtained from GenScript. DNA was heat shock-transformed into competent *E. coli* cells (ThermoFisher). Cells were streaked on agar plates and grown for 16 hours at 37°C. Thirteen bottles of 200 mL LB (Luria Broth) medium were prepared (MP Bio) and autoclaved. 300 µL ampicillin (100 mg/mL) was added to each bottle. Pre-cultures were started with 3 mL LB medium for each construct. After 6 hours, the pre-cultures were transferred into the bottles and grown for 16 hours at 37°C with agitation. DNA was purified from bacterial pellets by a plasmid DNA purification kit according the manufacturer's

instructions (MaxiPrep, NucleoBond PC 500, Macherey-Nagel) and reconstituted in TE buffer. Plasmid DNA concentration was determined by NanoDrop and was set to 1 µg/µL. The integrity of the plasmids was verified by restriction analysis. For each construct 1 µL plasmid DNA and 9 µL restriction enzyme-mix (PstI and PvuII) were mixed and incubated for 2 hours at 37°C. The DNA was analyzed on a 1% agarose gel after 1 hour running at 100 V using Bio-Rad ChemiDoc MP system. DNA constructs for the fine mapping (see below) were handled the same way but their integrity was checked by sequencing.

The mutant proteins were generated by transient transfection in HEK293T cells. HEK cells were cultured in complete DMEM (DMEM (Gibco) supplemented with 10% fetal calf serum (FCS) and 1% penicillin/streptomycin (P/S)). One day prior to transfection, cells of two flasks were seeded into fifteen 10 cm^2 culture dishes (Greiner Bio-One). Before the transfection, 21 mL of empty DMEM medium was mixed with 630 µL polyethylenimine (P-Pei, Polysciences, Inc.). As controls an empty plasmid PF45 pcDNA3.1 and PF146 H2B GFP were transfected. 15 µg plasmid DNA was incubated in 1500 µL empty medium-P-Pei mix for 20 minutes in Eppendorf-tubes. The transfection mix was carefully added to the cells and the medium was mixed by pipetting up and down. After 8 hours the medium was changed to 15 mL empty medium. After 3 days the cells were checked for GFP expression with a fluorescence microscope. Supernatants were collected on day 4 and were filter-sterilized by a 0.22 µm filter (Sartorius) and concentrated with a Vivaspin column (Sartorius) to approximately one-third of the original volume. Domain swap mutants were concentrated with 30,000 MWCO columns, and C2b mutants for fine mapping were concentrated with 10,000 MWCO columns. All supernatants were stored at -20°C and were analyzed also by SDS-PAGE and anti-FLAG Western Blot.

To verify expression of the various constructs, an anti-FLAG-tag ELISA assay was carried out. Microplates (Maxisorp, NUNC, Cat# 439454) were coated overnight with 100 µL of HEK293T supernatants 5x diluted in PBS or undiluted (for domain swap mutants and fine mapping mutants, respectively). After washing 4 times with PBS and 0.05% Tween-20, 100 µL/well of 1 µg/mL anti-FLAG Ab (clone M2, Sigma-Aldrich) in PBS and 0.1% Tween-20 (PBST) was added and incubated for 1 hour at room temperature (RT) with agitation. As detection Ab, 100 µL/well of horseradish peroxidase (HRP)-labeled goat anti-mouse-IgG (Santa Cruz Biotechnology, Cat# sc-2005, 1000x dil.) was added in PBST and incubated for 1 hour at RT. After a final washing step, 100 µL/well TMB (Invitrogen, Cat# SB02) was added as substrate, the reaction was stopped after a few minutes with 100 µL/well HCl

(Fischer, Cat# J/4320/15) and the absorbance was read at 450 nm (BioRad, iMark Microplate reader).

Anti-FLAG ELISA detected proteins in the supernatant for all mutants, except for C2-(FB-Pep1), as depicted in **Figure 11**. The variation between the mutants can be explained by the different production or by the different detection efficacy by anti-FLAG mAb after coating.

Next, the recognition of the swap mutants by the anti-C2-5F2.4 antibody was investigated. To this effect, microplates (Maxisorp, NUNC, Cat# 439454) were coated overnight with 2 µg/mL anti-C2-5F2.4 in 100 µL PBS. Plates were washed 4 times with PBS with 0.05% Tween-20 and blocked with 200 µL PBS with 0.1% Tween-20 with 1% bovine serum albumin (BSA) (PBST-BSA) for 1 hour at RT. After washing, 100 µL culture supernatant containing mutants were added 20x diluted in PBST-BSA and incubated for 2 hours at RT with agitation. After washing, as detection antibody 1 µg/mL biotinylated anti-FLAG (clone M2, Sigma-Aldrich) was added in PBST-BSA for 1 hour at RT. The plate was washed and 1 µg/mL streptavidin-POD conjugate (Roche, Cat# 11089153001) was added and incubated in the dark for 30 minutes. The plate was washed and 100 µL/well TMB (Invitrogen, Cat# SB02) was added as substrate, and reaction was stopped after a few minutes with 100 µL/well HCl (Fischer, Cat# J/4320/15). Absorbance was measured at 450 nm on a microplate reader (BioRad, iMark Microplate reader).

Wild type C2 showed clear binding, and loss of binding was only observed for C2-(FB-S2) in which the complement C2 S2 domain (SEQ ID NO: 46) was replaced by the Factor B S2 domain (SEQ ID NO: 54). In contrast, no binding was seen to wild type FB, however strong binding was detected for the mutant FB-(C2-S2) in which the Factor B S2 domain (SEQ ID NO: 54) was replaced by the complement C2 S2 domain (SEQ ID NO: 46), as depicted in **Figure 12**. These results clearly show that anti-C2-5F2.4 recognizes an epitope on S2 (Sushi domain 2) on C2b. This result also shows that C2-(FB-Pep1) is produced in sufficient quantity. Similar results were obtained when using the mouse IgG2a anti-C2-5F2.4. In addition, similar results were obtained when binding was studied in the presence of 1.25 mM Ca^{++} in the buffer. Epitope mapping performed by Bioceros BV, using domain swap mutants between human C2 and mouse C2, also led to a similar conclusion. Furthermore, the amino acid sequence of Sushi domain 2 of cynomolgus C2 is completely identical to Sushi domain 2 of human C2.

Example 7: Fine Mapping of Epitope of Anti-C2-5F2.4 within Sushi Domain 2

Anti-C2-5F2.4 does not cross-react with mouse C2, and the mouse S2 domain (SEQ ID NO: 58) differs from the human S2 domain (SEQ ID NO: 46) at 10 amino acid positions, as depicted in **Figure 13**. To investigate which of these ten amino acids is responsible for the mAb binding, fine mapping mutants were generated. The fine mapping constructs contained either the human C2b fragment (huC2b), huC2b with mouse S2 (huC2b-mS2), and ten mutants, each containing one amino acid back-mutation from the mouse sequence to the human sequence. Mutant C2b proteins were generated similar to the domain swap mutants by transient transfection into HEK293 cells.

All mutants were produced and detected by anti-FLAG ELISA, as depicted in **Figure 14**. Anti-C2-5F2.4 bound to huC2b but not to huC2b with a mouse S2 (huC2b-mS2), as expected. None of the reverse point mutations restored binding of anti-C2-5F2.4, suggesting that the epitope of this mAb is composed of at least two amino acids on the S2 domain, as depicted in **Figure 15**. Similar results were obtained when binding was studied in the presence of 1.25 mM Ca^{++} in the buffer.

By using the publicly available structural data for human C2b, the position of the ten possible amino acids that might contribute to the epitope of anti-C2-5F2.4 was analyzed, as depicted in **Figure 16**. This analysis revealed three possible clusters, each composed of three amino acids that could contribute to the epitope. DNA constructs for these cluster mutants were designed and obtained. The cluster mutants were generated by mutating the human C2b S2 amino acids to corresponding mouse C2b S2 amino acids. In each mutant three amino acids were changed. A loss of binding was expected if these three amino acids contributed to the epitope of anti-C2-5F2.4. **Figure 17A** shows that cluster 1 mutant was expressed well and the binding was not affected, and therefore these amino acids do not contribute to the binding. Based on the anti-FLAG ELISA, expression of the cluster 2 mutant was lower, and this resulted in lack of binding by anti-C2-5F2.4. Cluster 3 mutant also was not expressed well, and this most likely explains the lack of binding by anti-C2-5F2.4, as depicted in **Figure 17B**. Similar results were obtained when the binding was studied in the presence of 1.25 mM Ca^{++} in the buffer. From this analysis, the amino acids in cluster 1 can be excluded, leaving four possible amino acids in cluster 2 and cluster 3.

Domain swap mutants provided strong evidence that the epitope that is recognized by anti-C2-5F2.4 on C2 is located on the Sushi domain 2 on C2b. Additionally, these experiments suggest that the presence of that domain on FB is sufficient for recognition by

anti-C2-5F2.4. Considering that anti-C2-5F2.4 does not react with mouse C2, one or more of the 10 amino acids that differ between human and mouse Sushi 2 domain should be essential for the epitope. By using single amino acid back-mutations, we show that a single amino acid in Sushi 2 cannot restore binding. From the experiments performed with the cluster mutants it was concluded that amino acids in cluster 1 do not contribute to the epitope of anti-C2-5F2.4. Amino acids of cluster 2 may contribute to the epitope of anti-C2-5F2.4, but since the expression of this mutant was lower than cluster 1 mutant, it cannot be excluded that the folding of cluster 2 mutant was not optimal. Since the cluster 3 mutant was not well expressed, it appears likely the mutations affected folding and so the role of the amino acids in cluster 3 remains elusive.

Krav:

1. Monoklonalt antistof eller antigenbindende fragment deraf, som specifikt binder til human komplementfaktor C2, hvori nævnte monoklonale antistof eller nævnte antigenbindende fragment deraf omfatter:
et VH-domæne, som omfatter aminosyresekvensen angivet i SEQ ID NO: 3; og
et VL-domæne, som omfatter aminosyresekvensen angivet i SEQ ID NO: 2.

2. Monoklonalt antistof ifølge krav 1, hvori det monoklonale antistof omfatter et monoklonalt antistof i fuld længde.

3. Monoklonalt antistof eller det antigenbindende fragment deraf ifølge krav 1 eller krav 2, hvori det monoklonale antistof eller det antigenbindende fragment deraf omfatter en human IgG-tungkæde med konstant domæne.

4. Monoklonalt antistof eller det antigenbindende fragment deraf ifølge krav 3, hvori tungkæden med konstant domæne omfatter en human IgG1-tungkæde med konstant domæne, valgfrit hvori den humane IgG1-tungkæde med konstant domæne omfatter aminosyresekvensen angivet i SEQ ID NO: 29 eller
hvori den humane IgG1-tungkæde med konstant domæne omfatter aminosyresekvensen angivet i SEQ ID NO: 4.

5. Monoklonalt antistof eller det antigen-bindende fragment deraf ifølge krav 3 eller krav 4, hvori tungkæden med konstant domæne omfatter aminosyresubstitutionerne L234A og L235A ifølge EU-nummereringsskemaet, og/eller
hvori tungkæden med konstant domæne omfatter aminosyresubstitutionerne H433K og N434F, ifølge EU-nummereringsskemaet.

6. Monoklonalt antistof eller det antigenbindende fragment deraf ifølge krav 3, hvori tungkæden med konstant domæne omfatter en human IgG4-tungkæde med konstant domæne, valgfrit hvori den humane IgG4-tungkæde med konstant domæne omfatter aminosyresekvensen angivet i SEQ ID NO: 5.

7. Monoklonalt antistof eller det antigenbindende fragment deraf ifølge krav 1 eller krav 3, eller det monoklonale antistof ifølge krav 2, hvori det monoklonale antistof eller antigenbindende fragment deraf omfatter en tungkæde omfattende aminosyresekvensen

angivet som SEQ ID NO: 6 og en letkæde omfattende aminosyresekvensen angivet som SEQ ID NO: 7 eller
hvori det monoklonale antistof eller antigenbindende fragment deraf omfatter en tungkæde omfattende aminosyresekvensen angivet som SEQ ID NO: 8 og en letkæde omfattende aminosyresekvensen angivet som SEQ ID NO: 7.

8. Nukleinsyremolekyle eller flerhed af nukleinsyremolekyler, som koder for det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller det monoklonale antistof ifølge krav 2.

9. Vektor eller flerhed af vektorer omfattende nukleinsyremolekylet eller flerheden af nukleinsyremolekyler ifølge krav 8.

10. Vektor eller flerhed af vektorer ifølge krav 9, hvori vektoren eller flerheden af vektorer er en virusvektor, valgfrit hvori virusvektoren er en adenovirusvektor, en lentivirusvektor, en adeno-associeret virusvektor eller en retrovirusvektor.

11. Værtscelle omfattende:
(a) nukleinsyremolekylet eller flerheden af nukleinsyremolekyler ifølge krav 8;
(b) vektoren eller flerheden af vektorer ifølge krav 9 eller krav 10;
(c) et første nukleinsyremolekyle, som koder for en tungkæde med variabel region eller en tungkæde af det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller det monoklonale antistof ifølge krav 2 og et anden nukleinsyremolekyle, som koder for en letkæde med variabel region eller en letkæde af det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller det monoklonale antistof ifølge krav 2; eller
(d) en første vektor omfattende et første polynukleotid, som koder for en tungkæde med variabel region eller en tungkæde af det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller det monoklonale antistof ifølge krav 2 og en anden vektor, som omfatter et anden polynukleotid, som koder for en letkæde med variabel region eller letkæde af det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller det monoklonale antistof ifølge krav 2.

12. Værtscelle ifølge krav 11, hvori værtscellen er en pattedyrcelle, valgfrit hvori værtscellen er en hybridomcelle, en kinesisk hamster-ovarie (CHO) celle, en NS0-celle, eller en human embryonisk nyrecelle (HEK293).

13. Fremgangsmåde til fremstilling af et monoklonalt antistof eller antigenbindende fragment deraf, omfattende
at dyrke en population af værtsceller ifølge krav 11 eller krav 12 under betingelser, som er egnede til ekspression af det monoklonale antistof eller antigenbindende fragment deraf; og
at isolere det monoklonale antistof eller antigenbindende fragment deraf fra cellerne.

14. Farmaceutisk sammensætning omfattende det monoklonale antistof eller det antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, det monoklonale antistof ifølge krav 2, nukleinsyremolekylet eller flerheden af nukleinsyremolekyler ifølge krav 8, vektoren eller flerheden af vektorer ifølge krav 9 eller krav 10, eller værtscellen ifølge krav 11 eller krav 12, og en farmaceutisk acceptabel bærer.

15. Monoklonalt antistof eller antigenbindende fragment deraf ifølge ethvert af kravene 1 eller 3 – 7, eller et monoklonalt antistof ifølge krav 2, eller en farmaceutisk sammensætning ifølge krav 14 til anvendelse i en fremgangsmåde til inhibering af den klassiske vej eller lectinvej til komplementaktivering i et individ og behandling eller forebyggelse af en sygdom eller tilstand valgt blandt eksperimentel allergisk neuritis, type II kollagen-induceret arthritis, myasthenia gravis, hæmolytisk anæmi, glomerulonephritis, idiopatisk membranøs nefropati, reumatoid arthritis, systemisk lupus erythematosus, immunkompleksinduceret vaskulitis, multipel sklerose, respiratory distress syndrome hos voksne, slagtilfælde, xenotransplantation, allotransplantation, forbrændingsskader, sepsis, septisk chok, toksicitet induceret af *in vivo* administrationen af cytokiner eller mAbs, antistof-medieret afstødning af allotransplantater, såsom nyre-allotransplantater, multiple traumer, iskæmi-reperfusionsskader og myokardieinfarkt.

16. Monoklonalt antistof, antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 15, hvori individet er et menneske.

17. Monoklonalt antistof, antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 15 eller krav 16, hvori det monoklonale antistof, antigenbindende fragment deraf eller farmaceutisk sammensætning er til intravenøs administration til individet.

18. Monoklonalt antistof, antigenbindende fragment deraf eller farmaceutisk sammensætning til anvendelse ifølge krav 15 eller krav 16, hvori det monoklonale

antistof, antigenbindende fragment deraf eller farmaceutisk sammensætning er til subkutan administration til individet.

DRAWINGS

Drawing

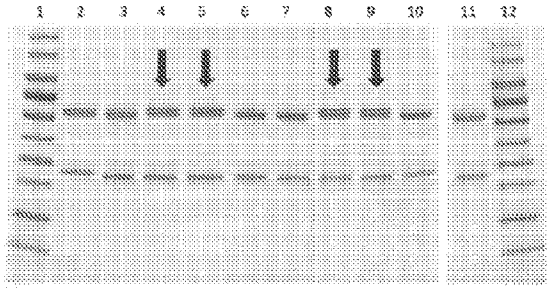

Fig. 1

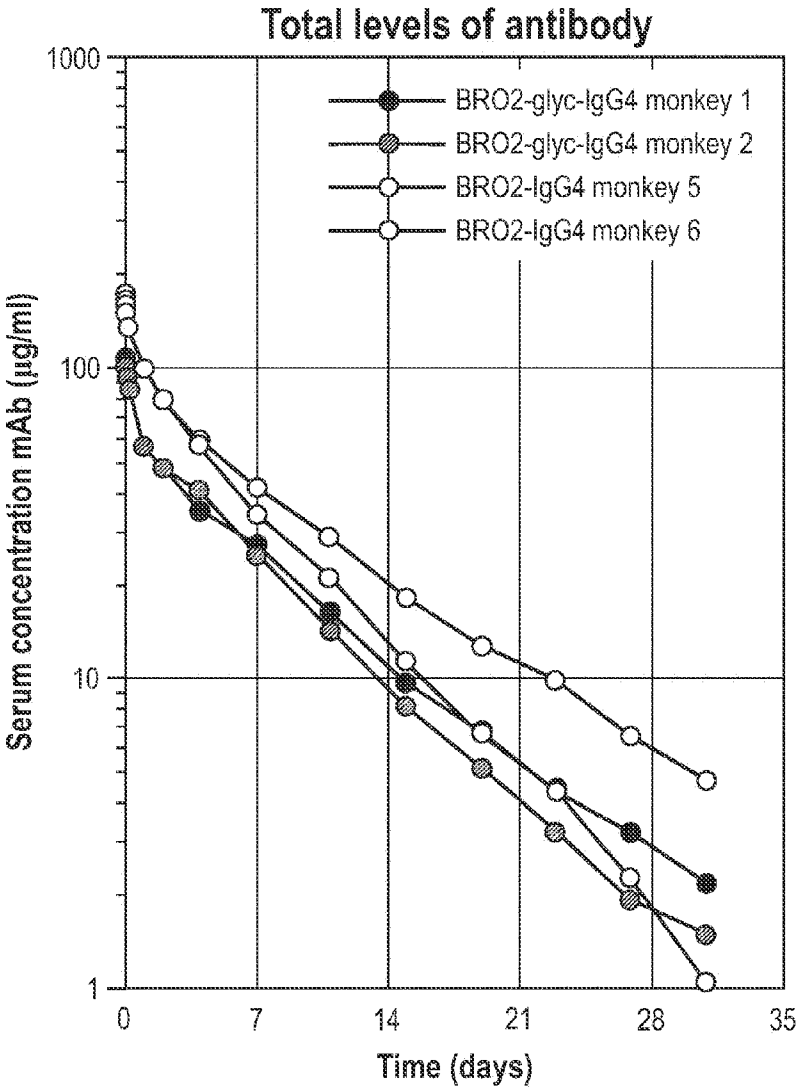
Total levels of antibody
BRO2-glyc-IgG4 monkey 1
BRO2-glyc-IgG4 monkey 2
BRO2-IgG4 monkey 5
BRO2-IgG4 monkey 6
Serum concentration mAb (µg/ml)
1000
100
10
1
0
7
14
21
28
35
Time (days)

Fig. 2

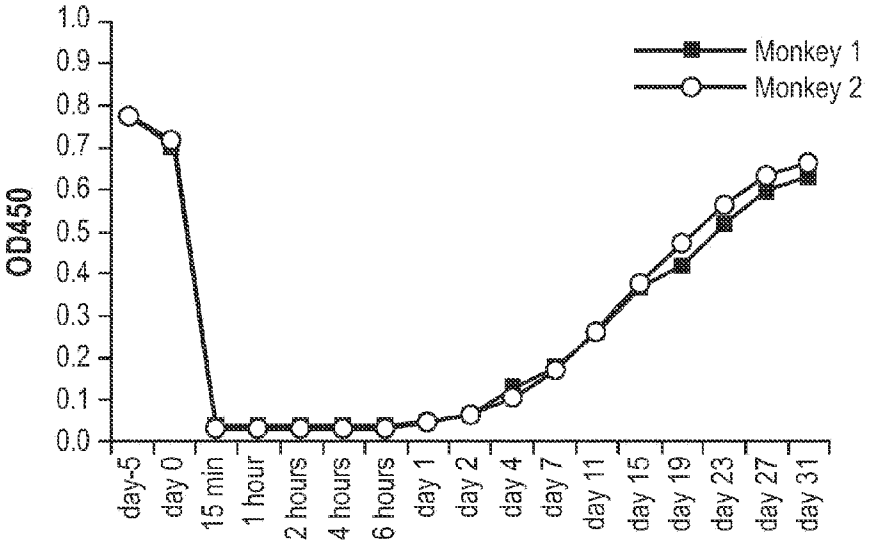
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 1
Monkey 2
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3A

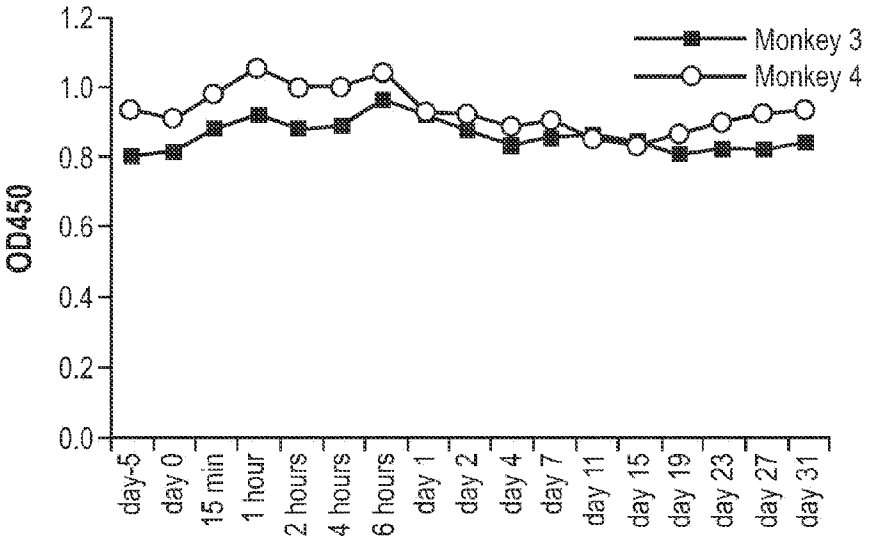
OD450
1.2
1.0
0.8
0.6
0.4
0.2
0.0
Monkey 3
Monkey 4
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3B

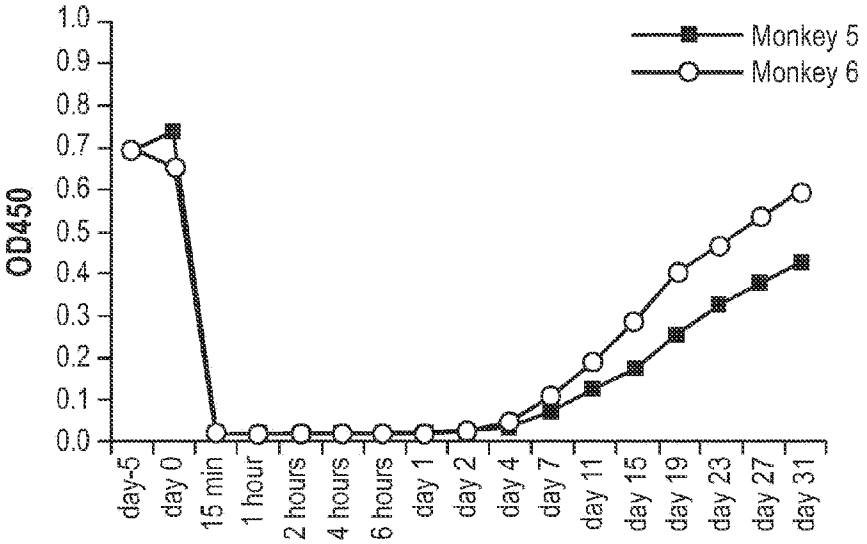
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 5
Monkey 6
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3C

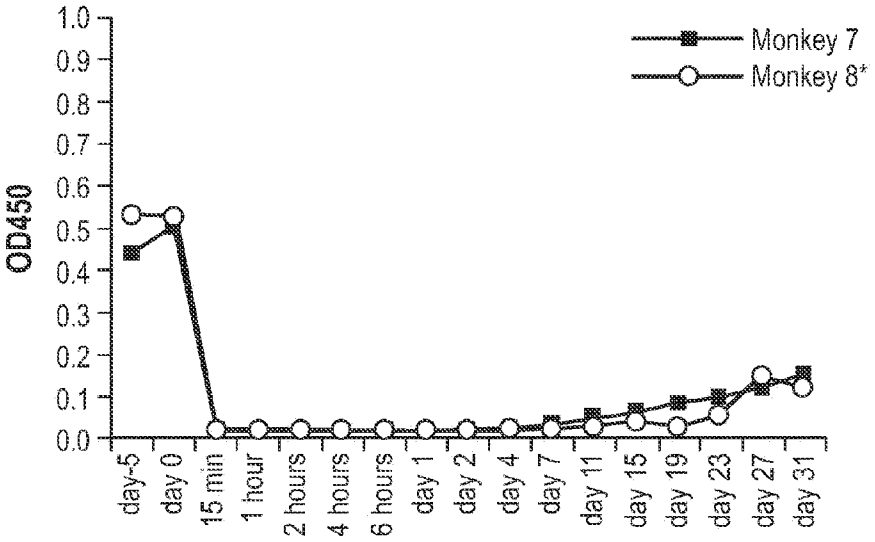
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 7
Monkey 8*
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3D

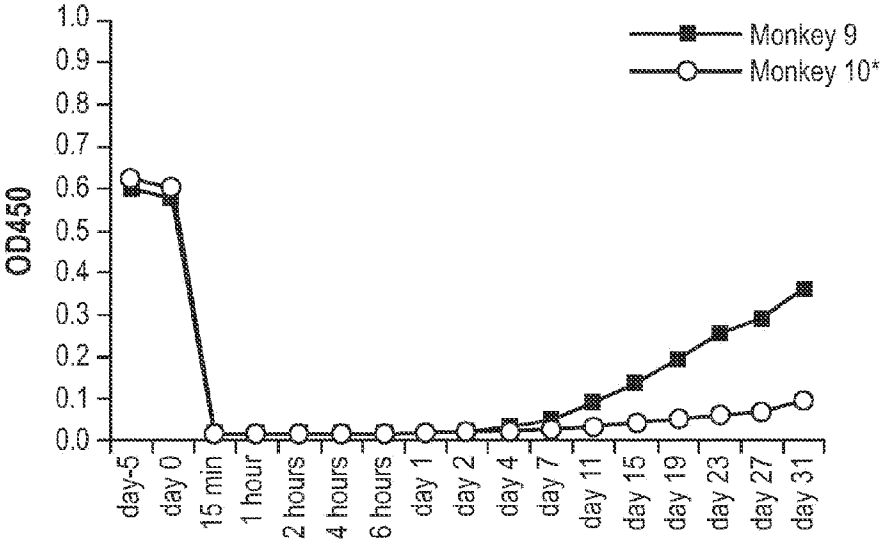
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 9
Monkey 10*
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3E

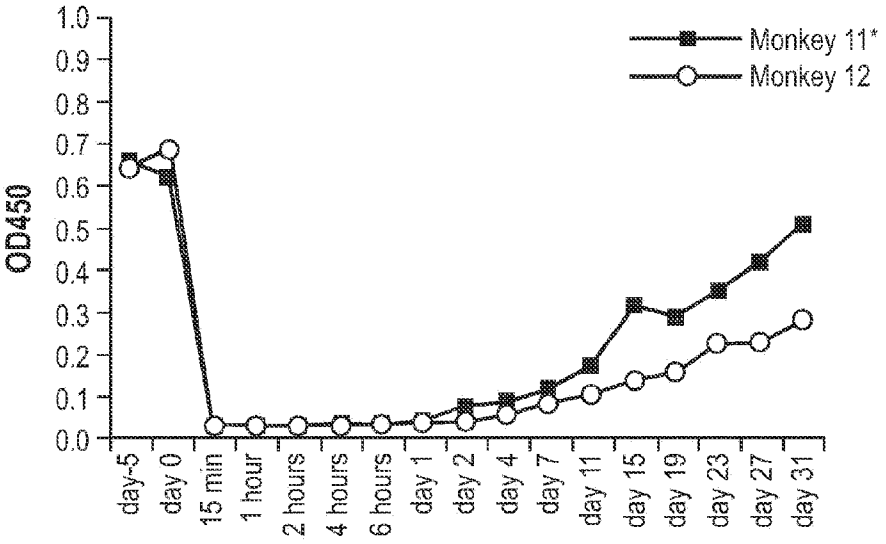
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 11*
Monkey 12
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3F

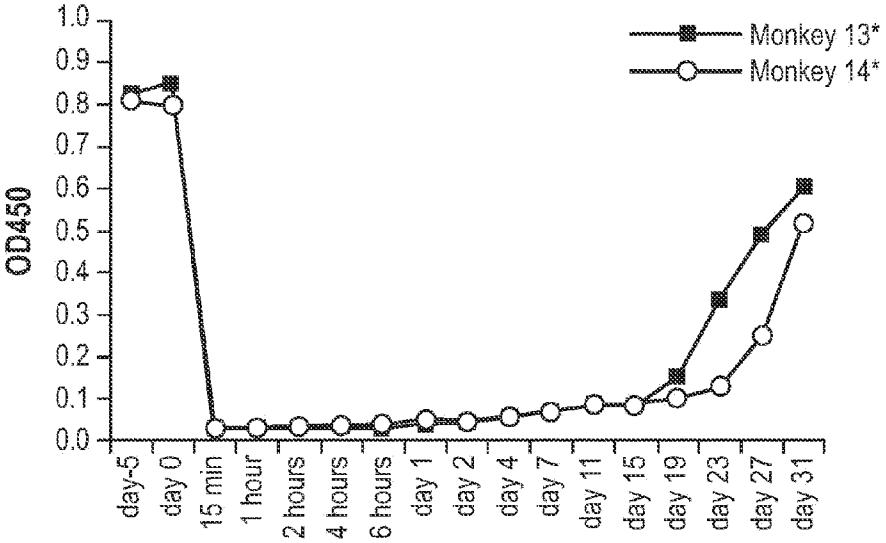
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 13*
Monkey 14*
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3G

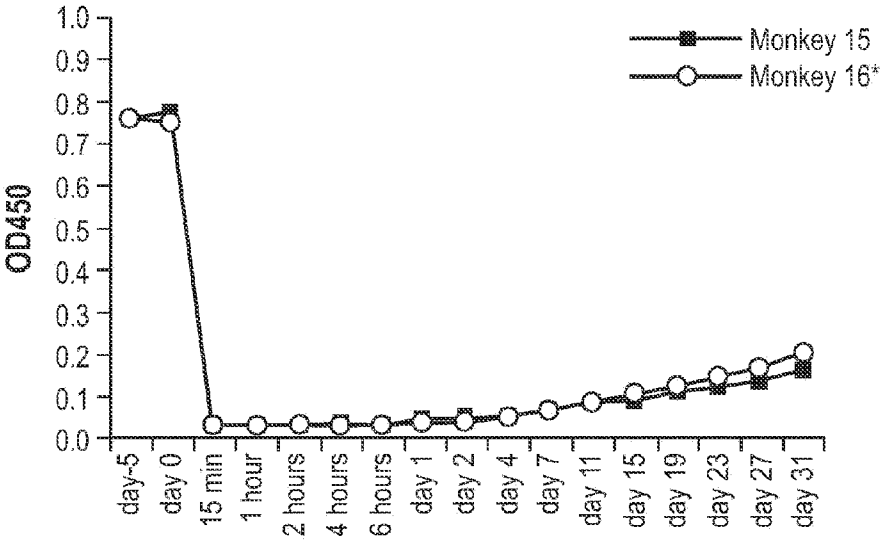
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
Monkey 15
Monkey 16*
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 3H

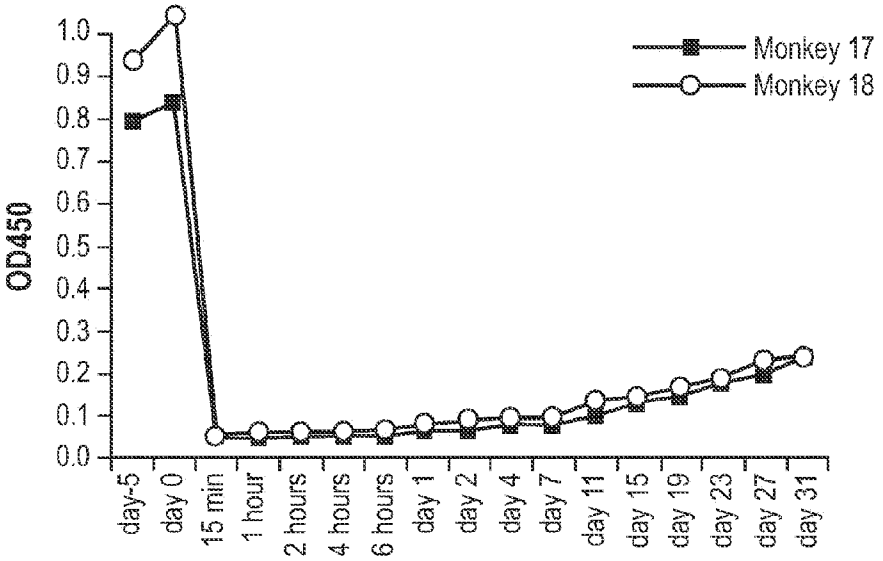
OD450
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
day-5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31
Monkey 17
Monkey 18

Fig. 3I

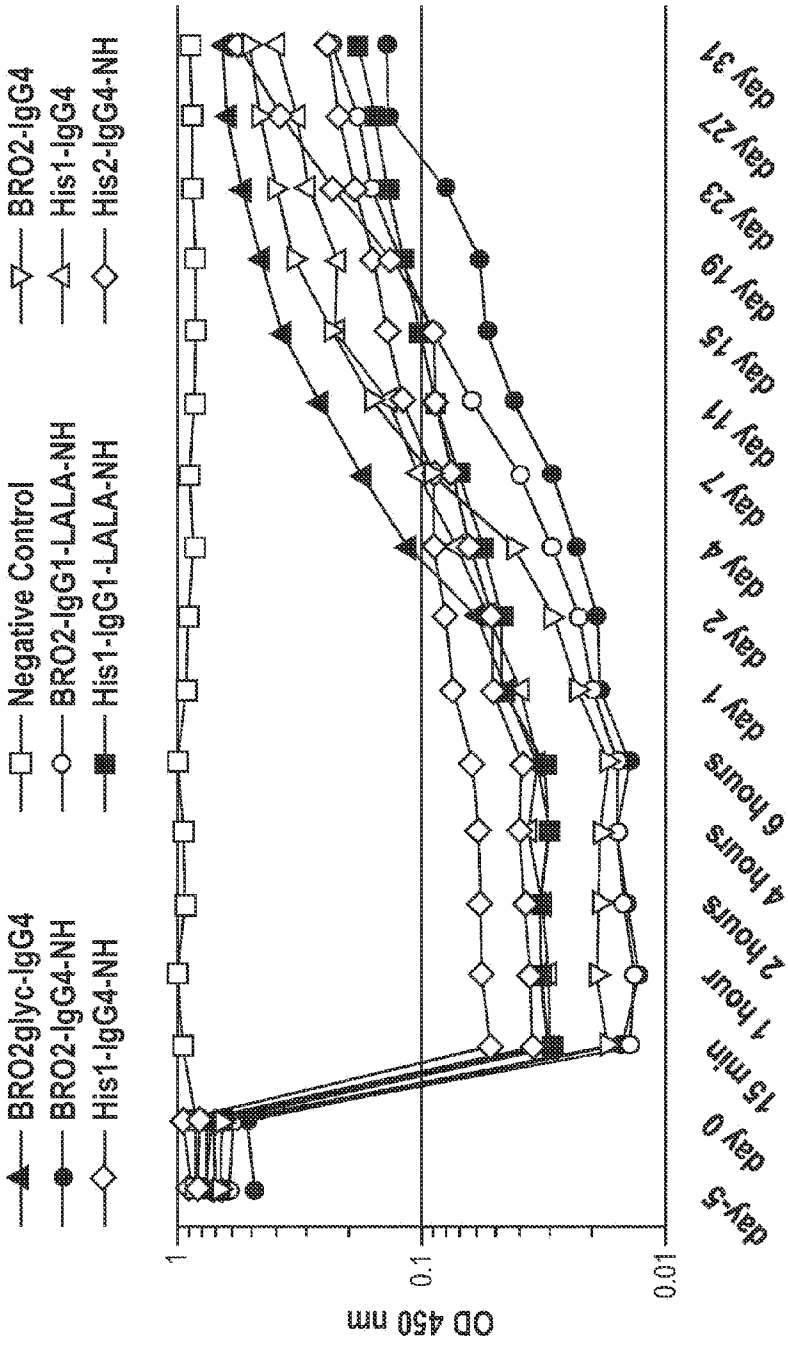
BRO2glyc-IgG4
BRO2-IgG4-NH
His1-IgG4-NH
Negative Control
BRO2-IgG1-LALA-NH
His1-IgG1-LALA-NH
BRO2-IgG4
His1-IgG4
His2-IgG4-NH
OD 450 nm
1
0.1
0.01
day -5
day 0
15 min
1 hour
2 hours
4 hours
6 hours
day 1
day 2
day 4
day 7
day 11
day 15
day 19
day 23
day 27
day 31

Fig. 4

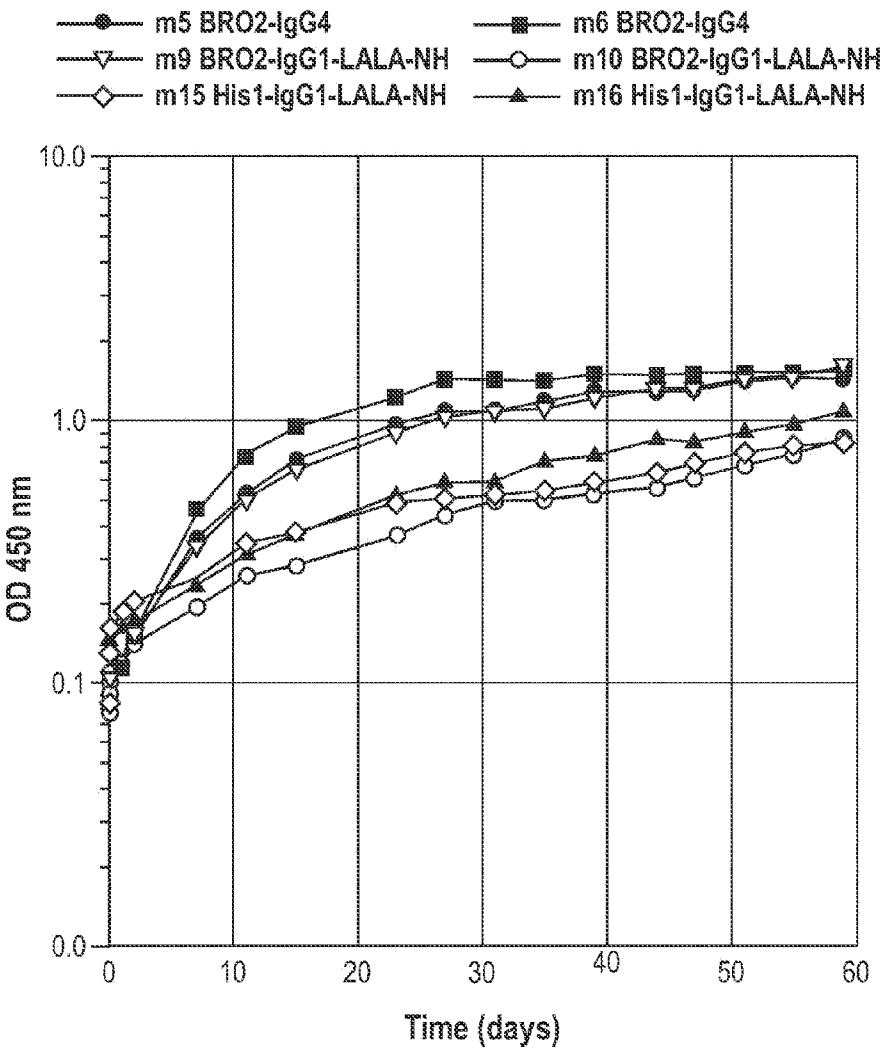
m5 BRO2-IgG4
m6 BRO2-IgG4
m9 BRO2-IgG1-LALA-NH
m10 BRO2-IgG1-LALA-NH
m15 His1-IgG1-LALA-NH
m16 His1-IgG1-LALA-NH
10.0
1.0
0.1
0.0
OD 450 nm
0
10
20
30
40
50
60
Time (days)

Fig. 5

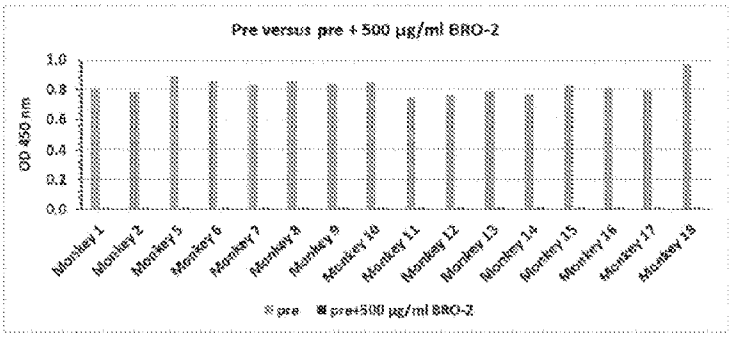

Fig. 6A

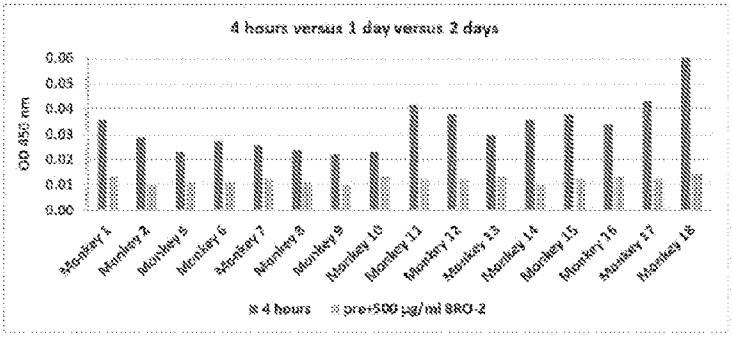

Fig. 6B

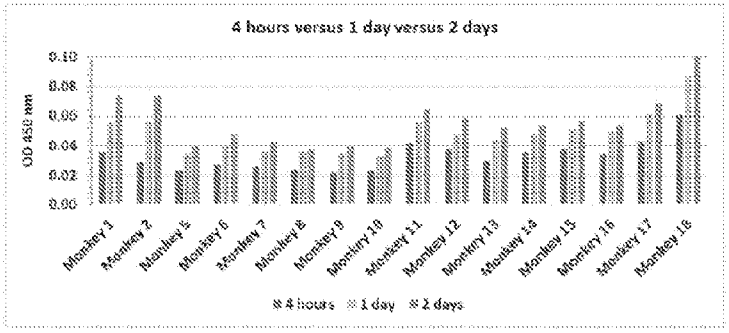

Fig. 6C

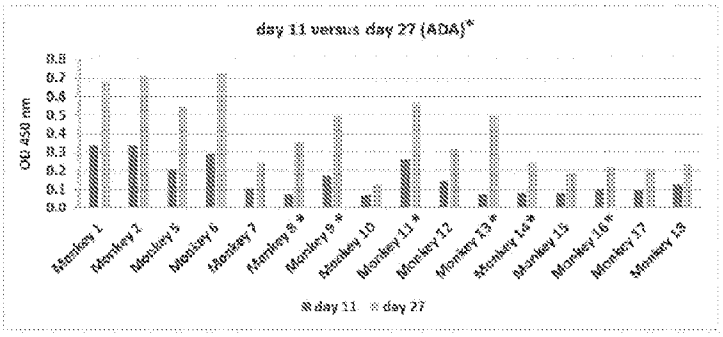
day 11 versus day 27 (ADA)*
OD 450 nm
8.8
8.7
8.6
8.5
8.4
8.3
8.2
8.1
8.0
Monkey 1
Monkey 2
Monkey 5
Monkey 6
Monkey 7
Monkey 8 *
Monkey 9 *
Monkey 10
Monkey 11 *
Monkey 12
Monkey 13 *
Monkey 14 *
Monkey 15
Monkey 16 *
Monkey 17
Monkey 18
day 11
day 27

Fig. 6D

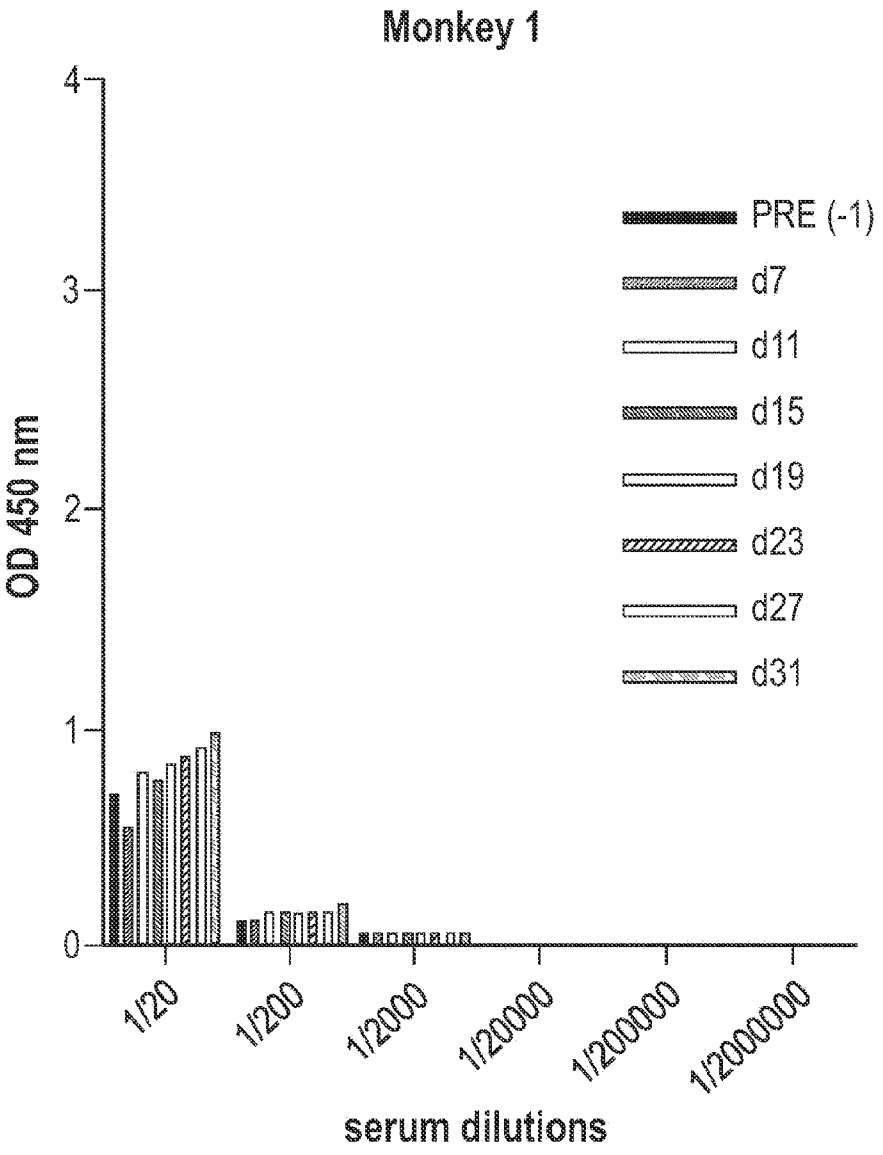
Monkey 1
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7A

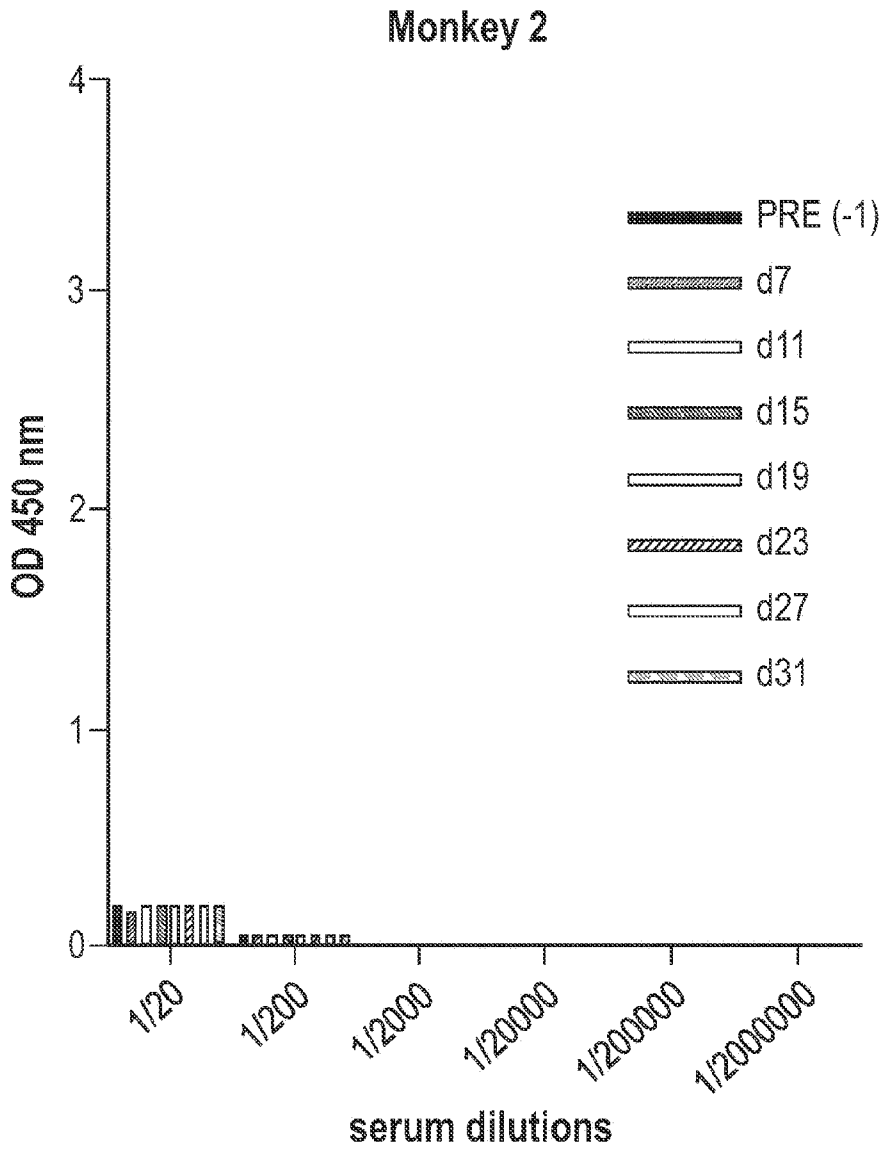
Monkey 2
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7B

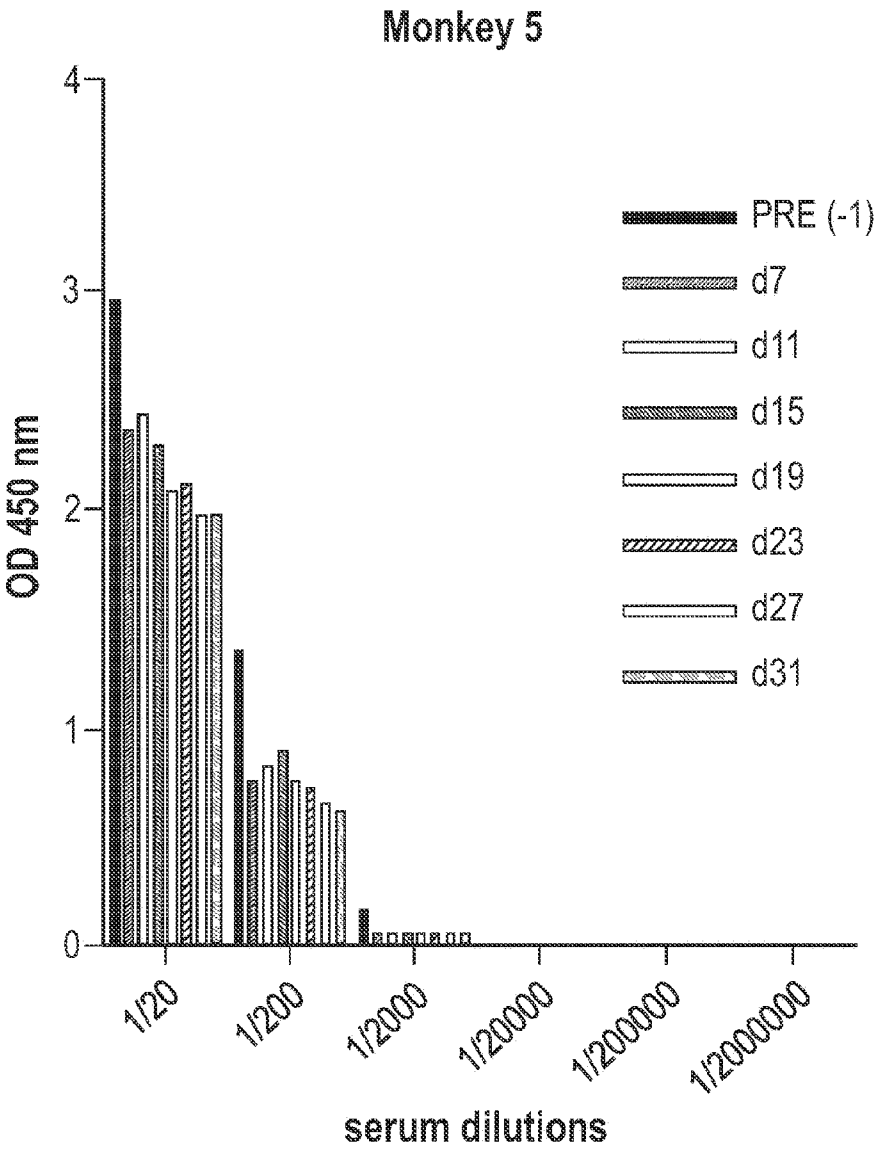
Monkey 5
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7C

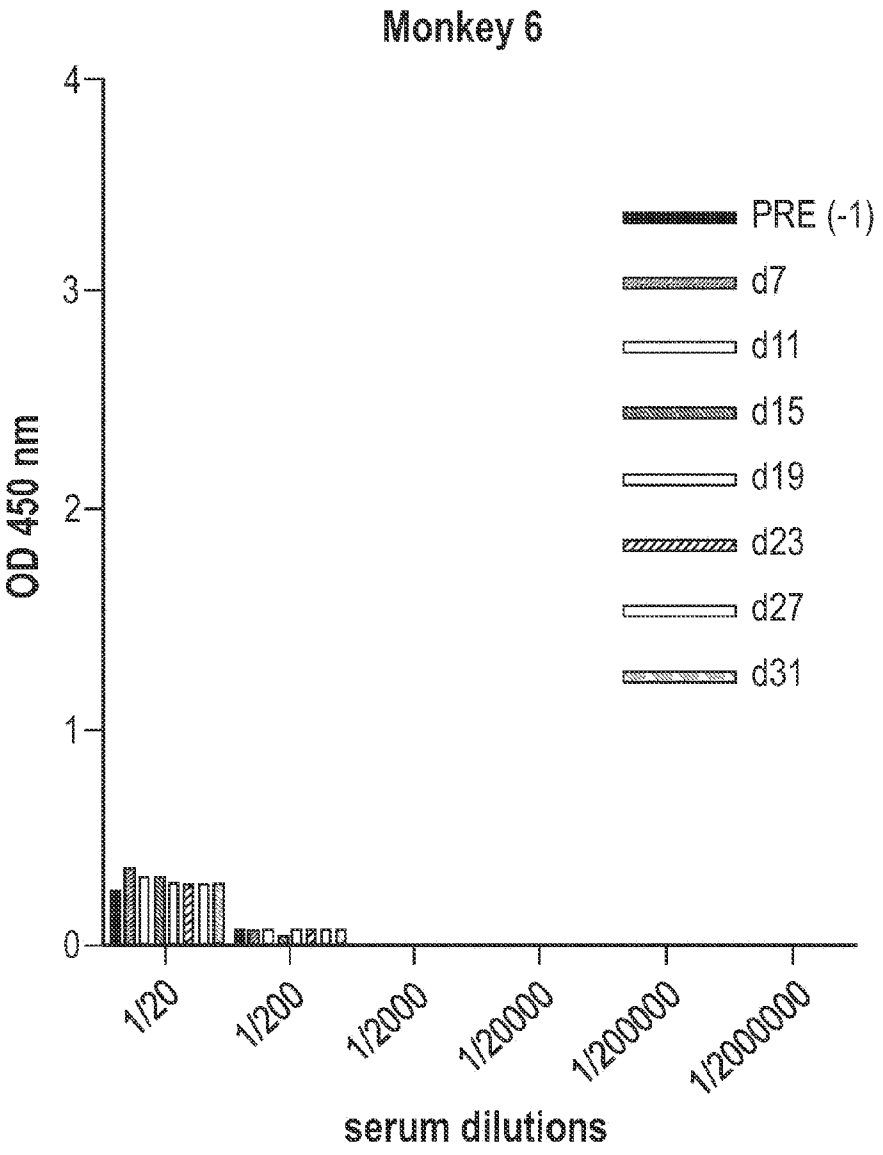
Monkey 6
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7D

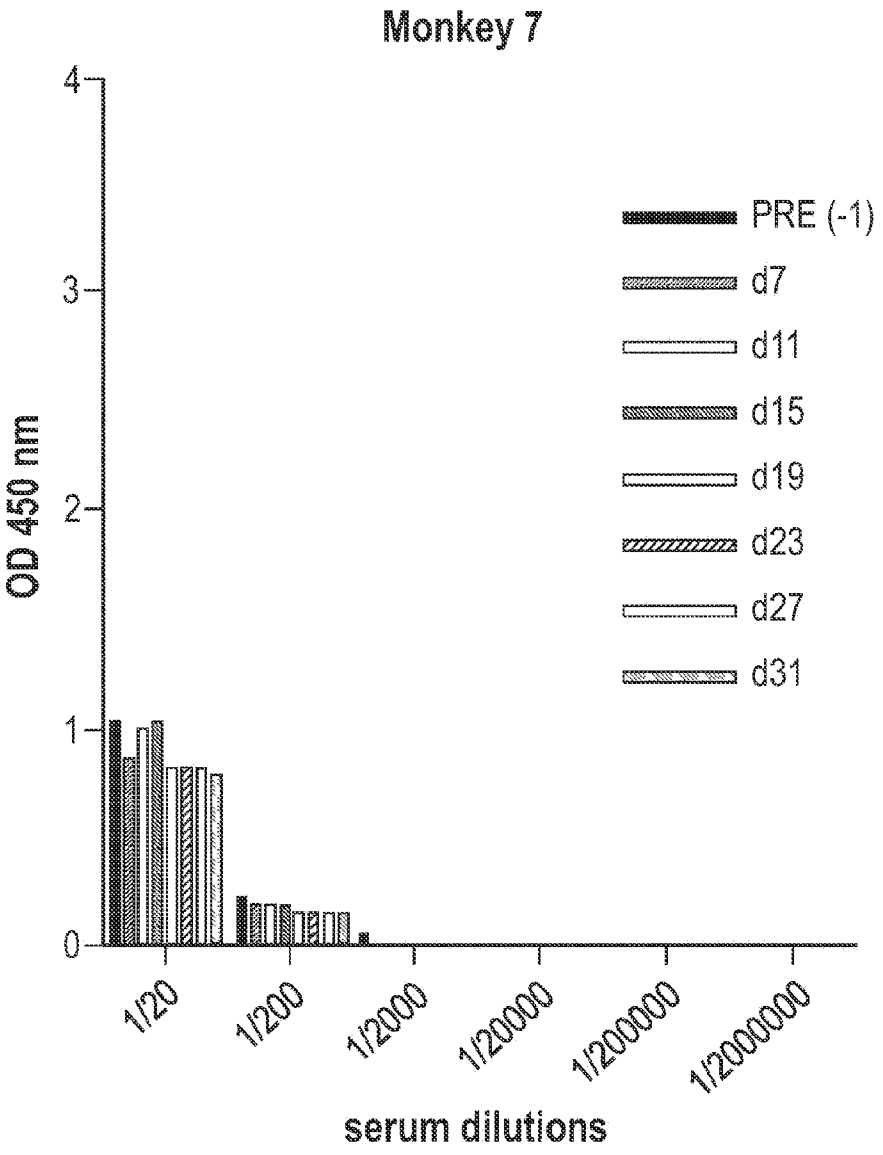
Monkey 7
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7E

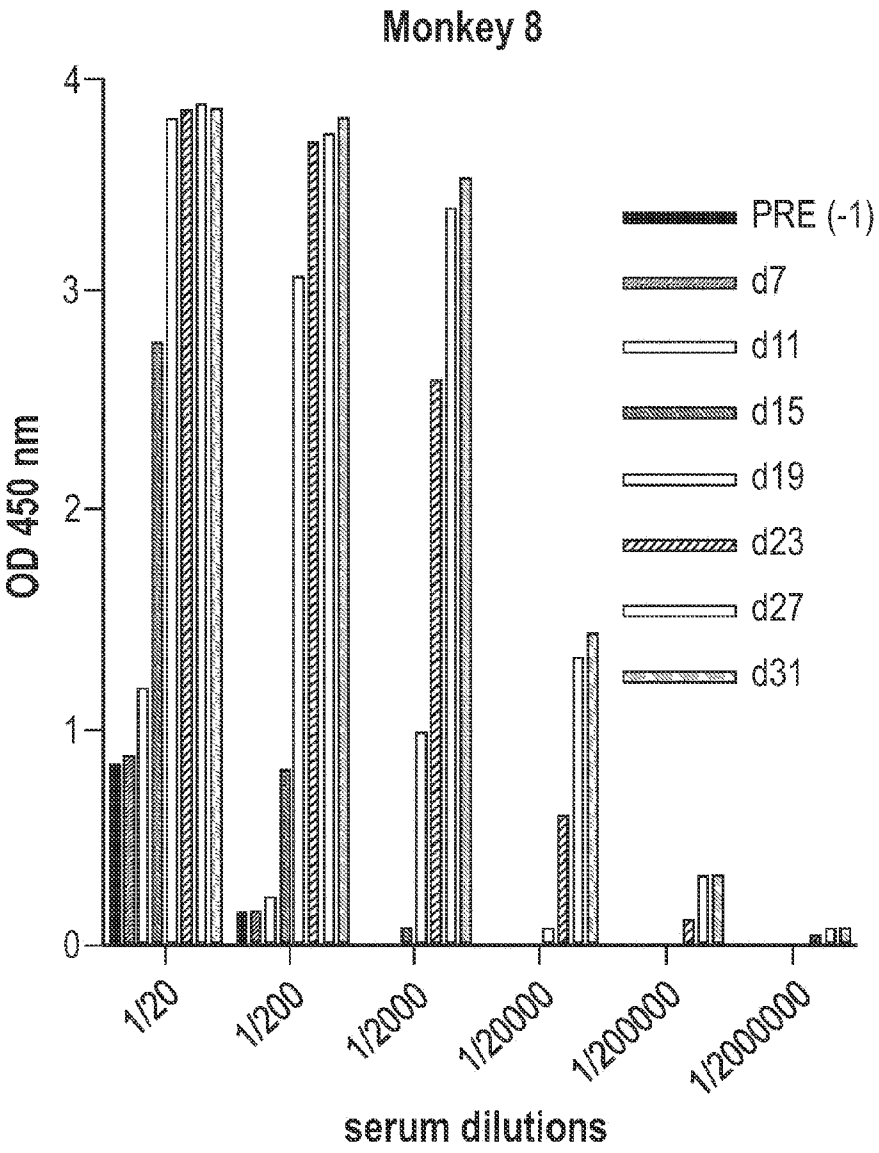
Monkey 8
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7F

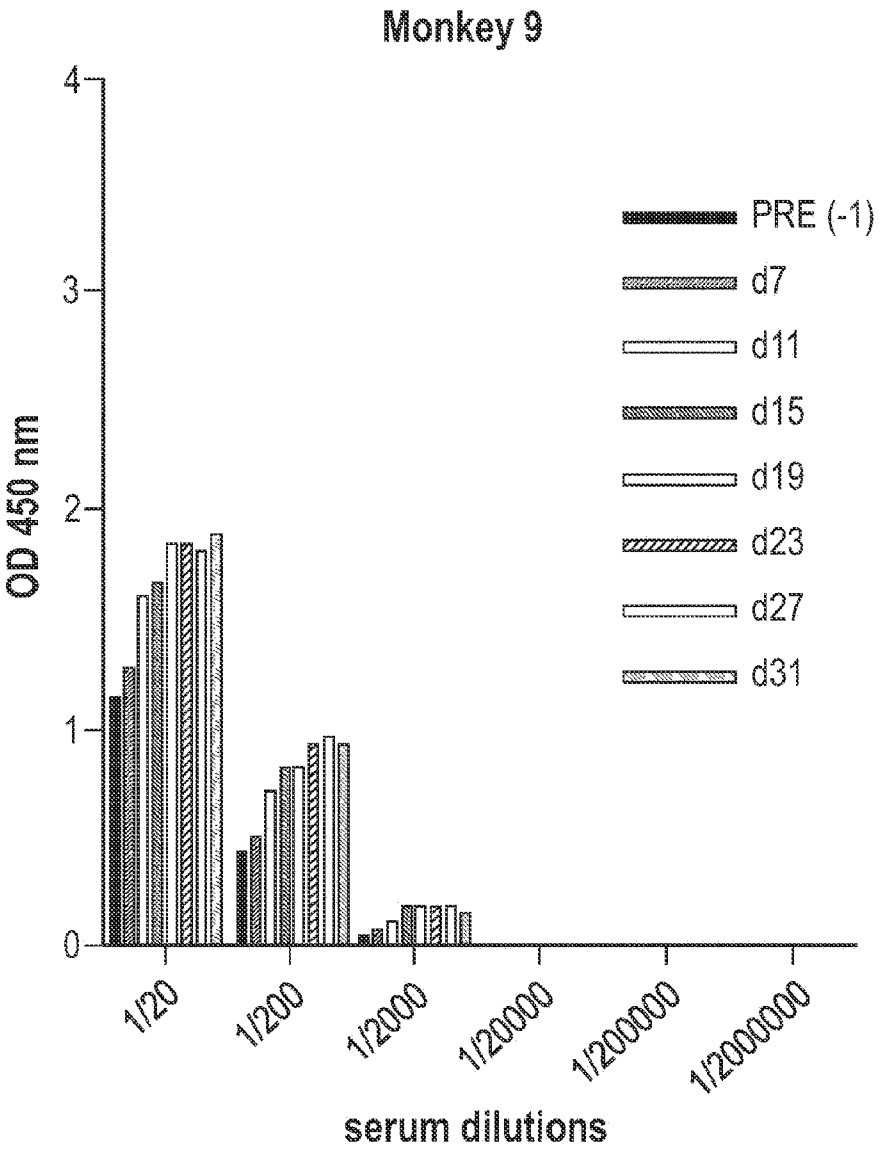
Monkey 9
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7G

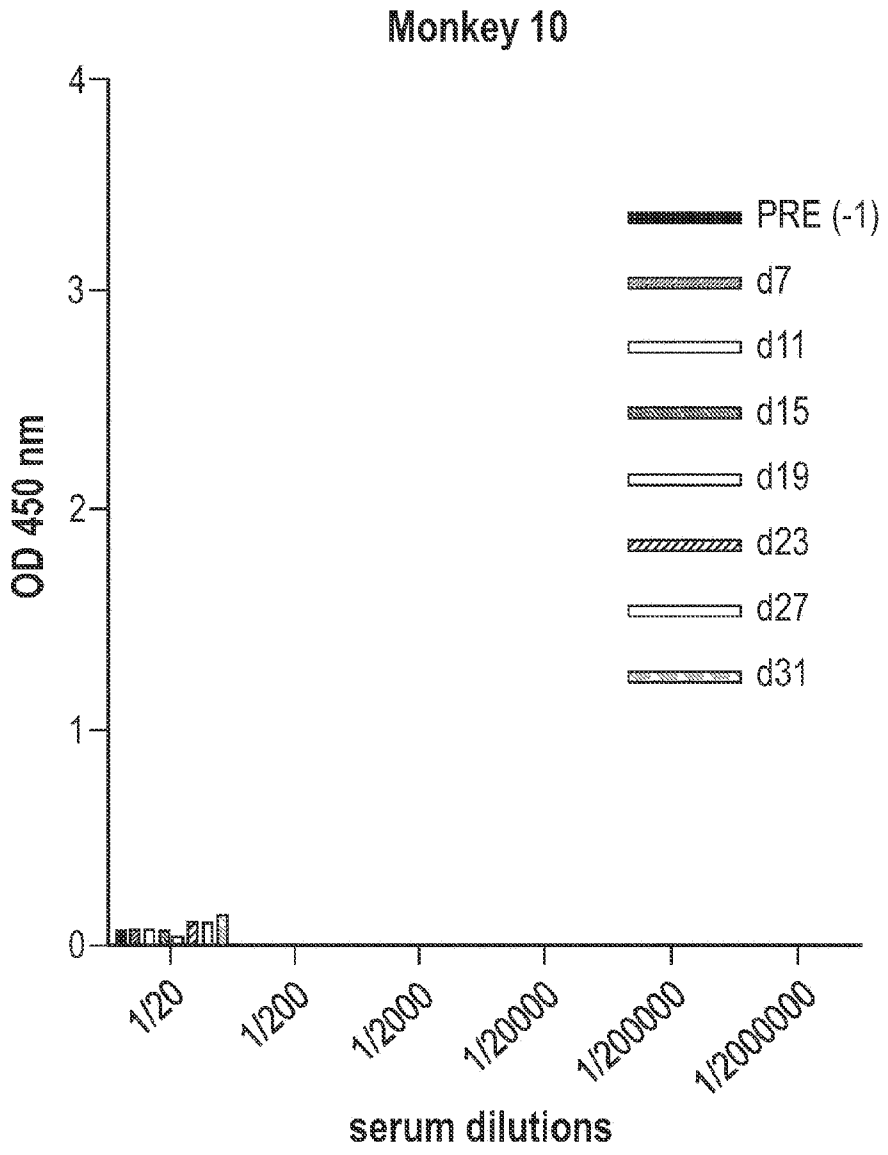
Monkey 10
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7H

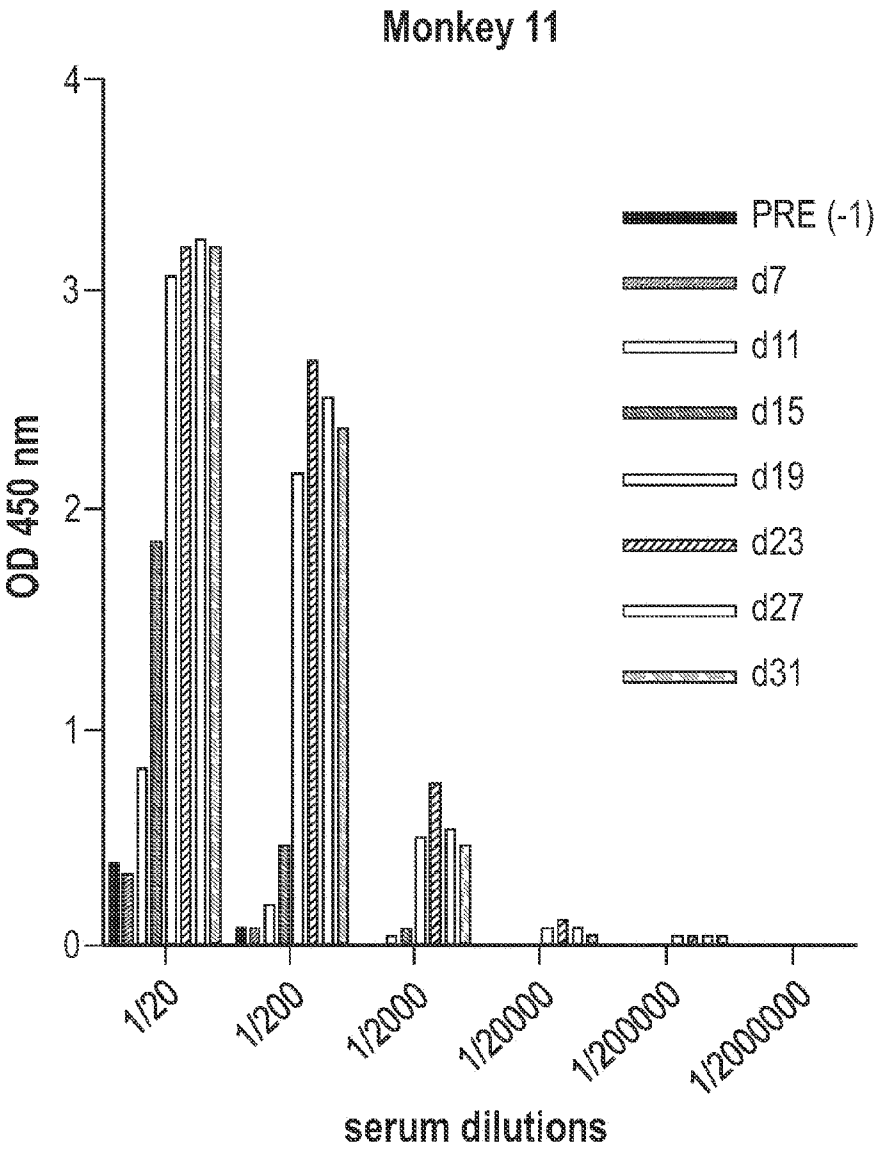
Monkey 11
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7I

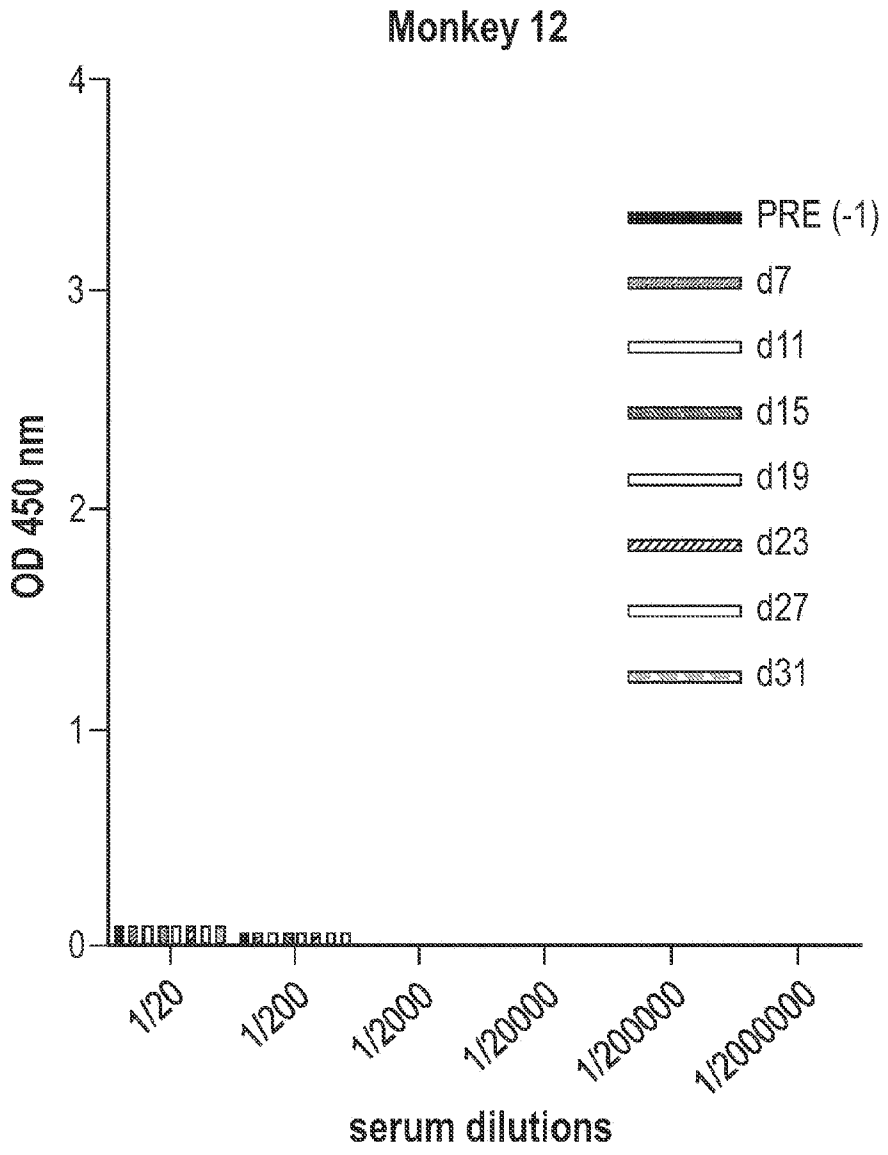
Monkey 12
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7J

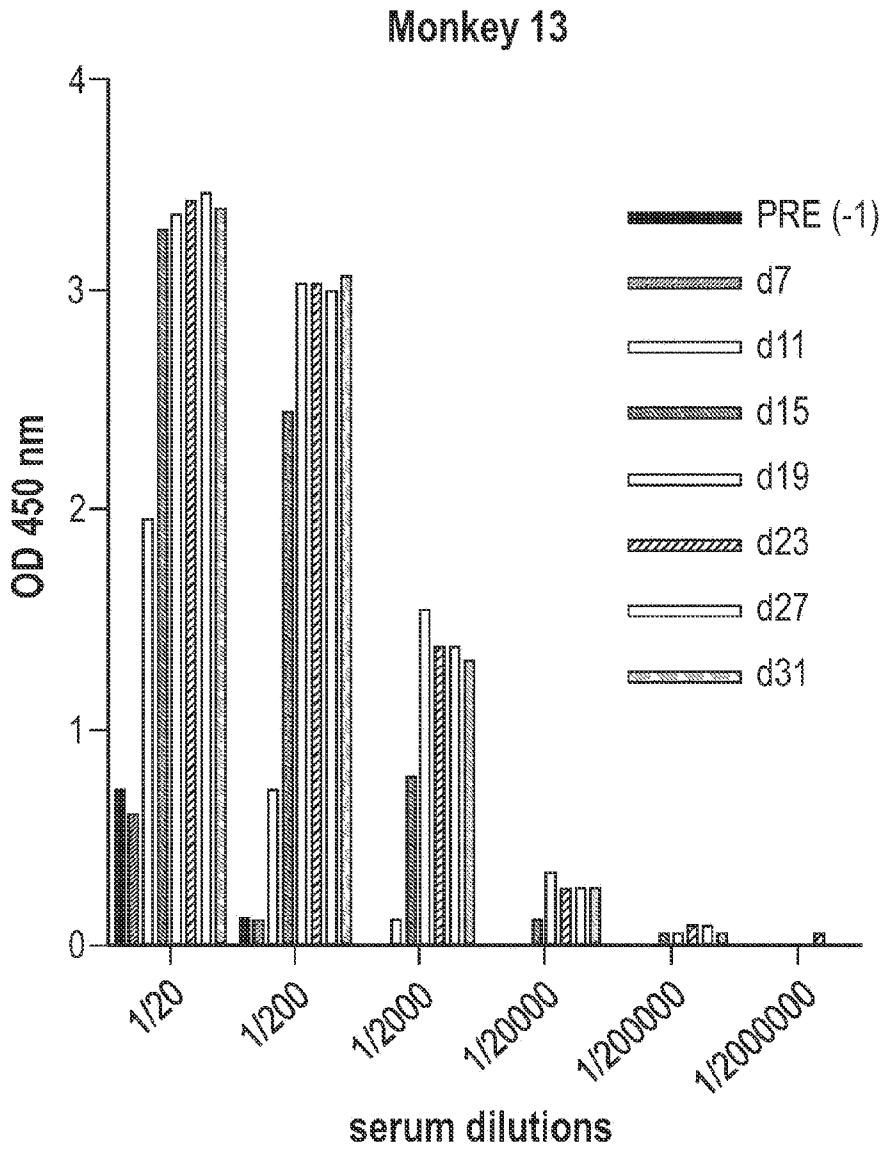
Monkey 13
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7K

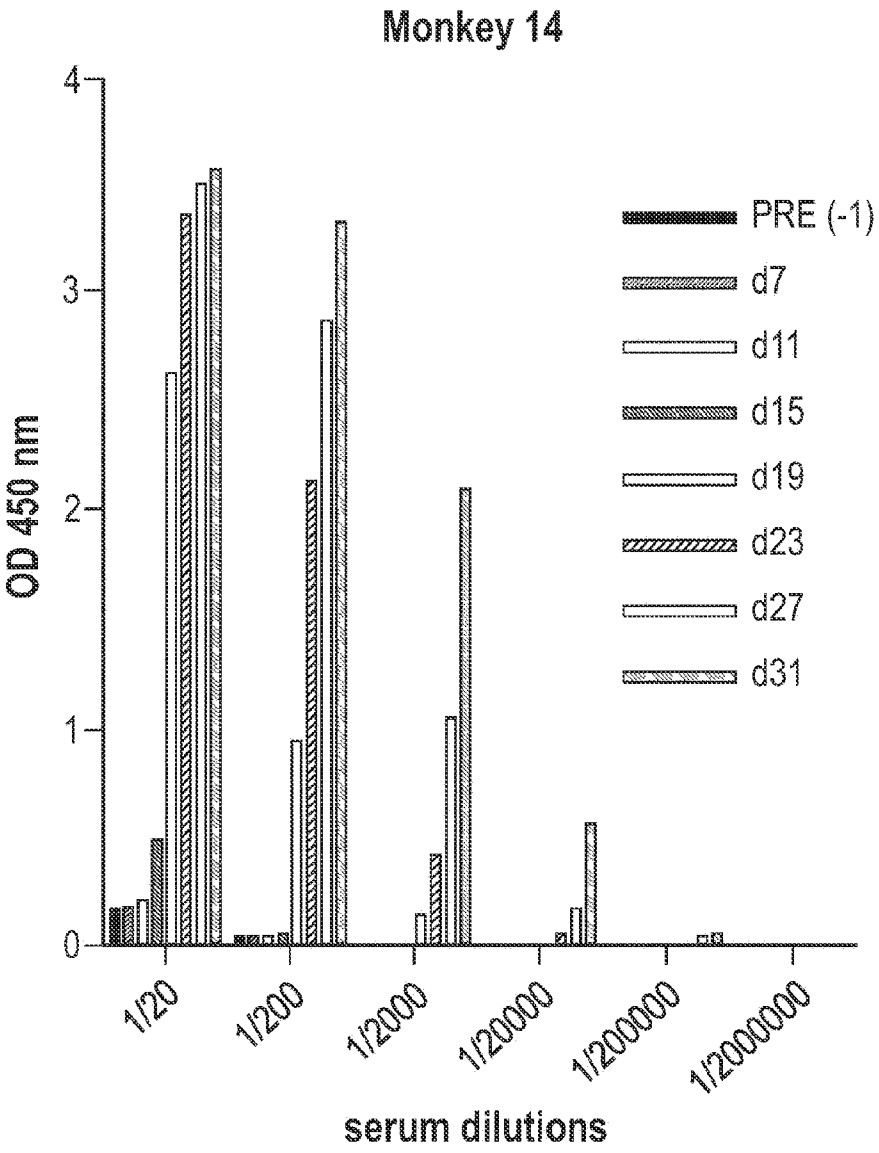
Monkey 14
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7L

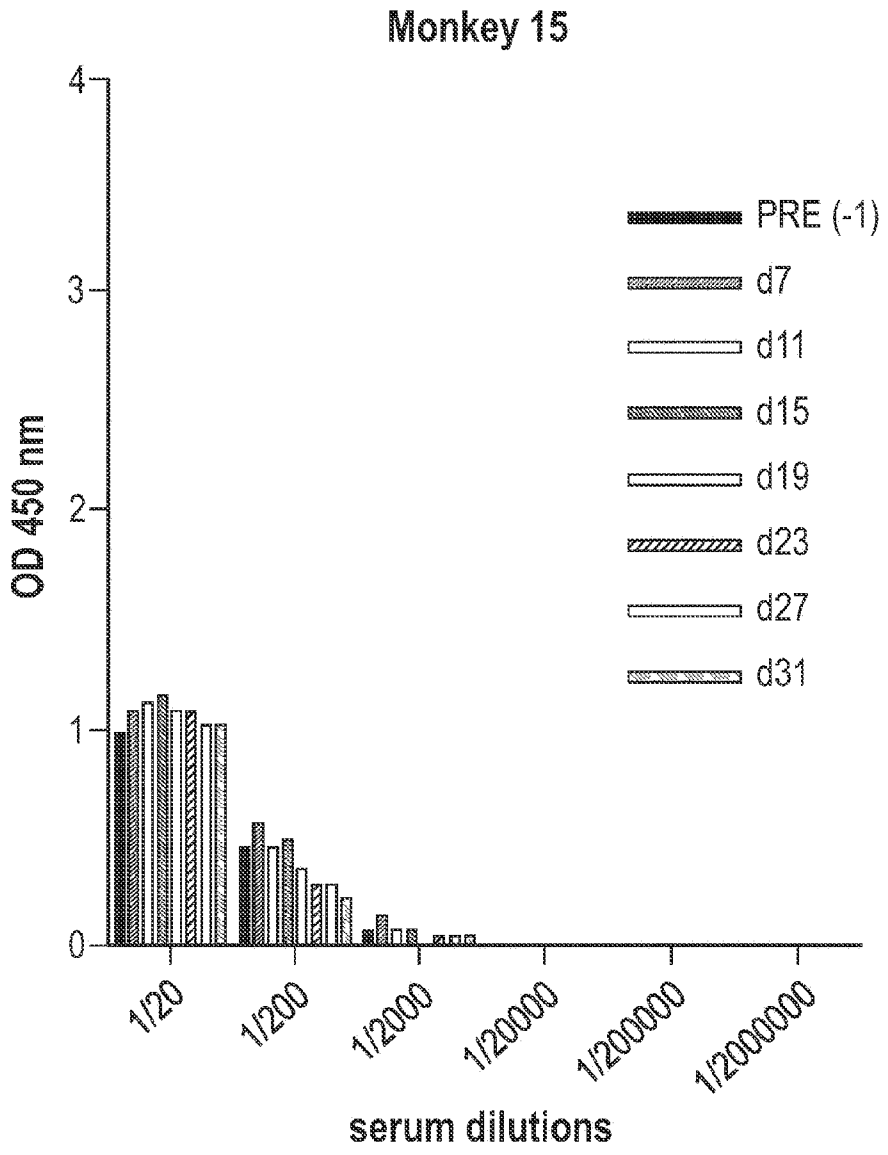
Monkey 15
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7M

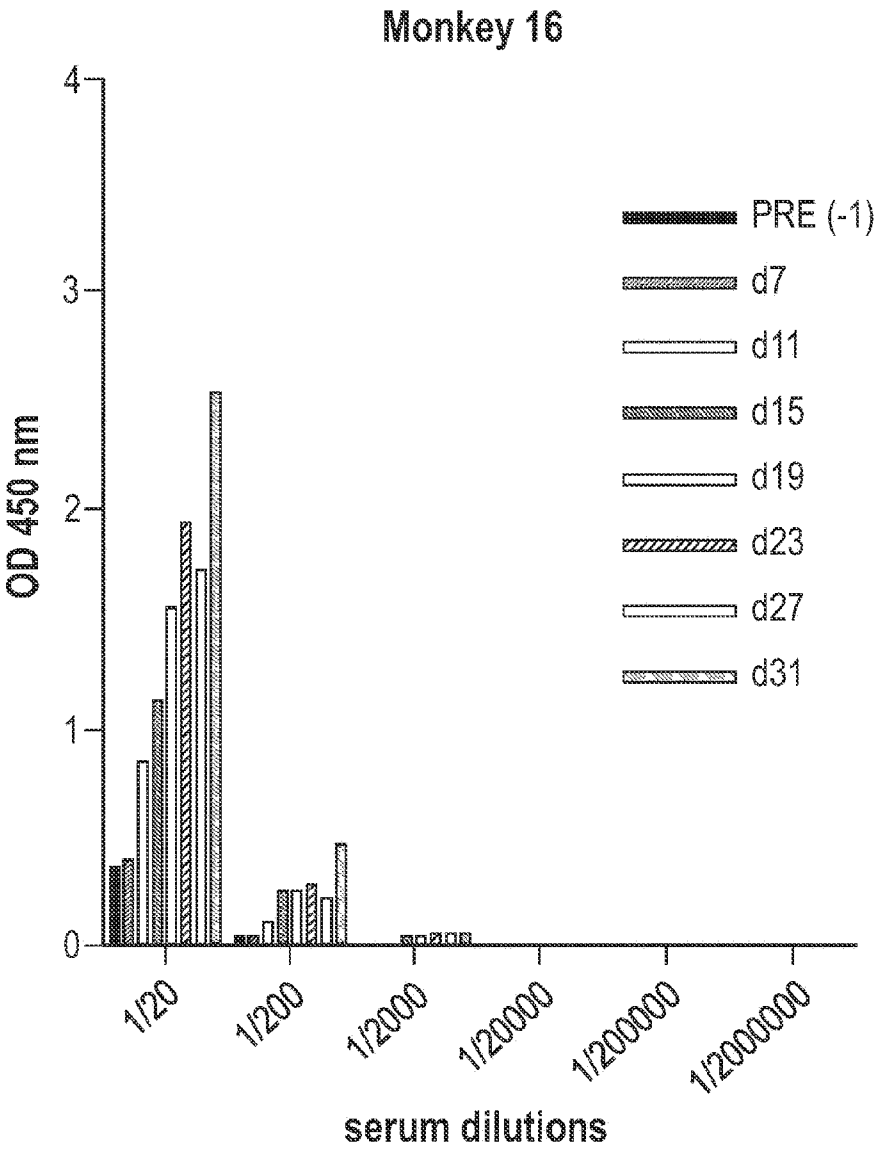
Monkey 16
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7N

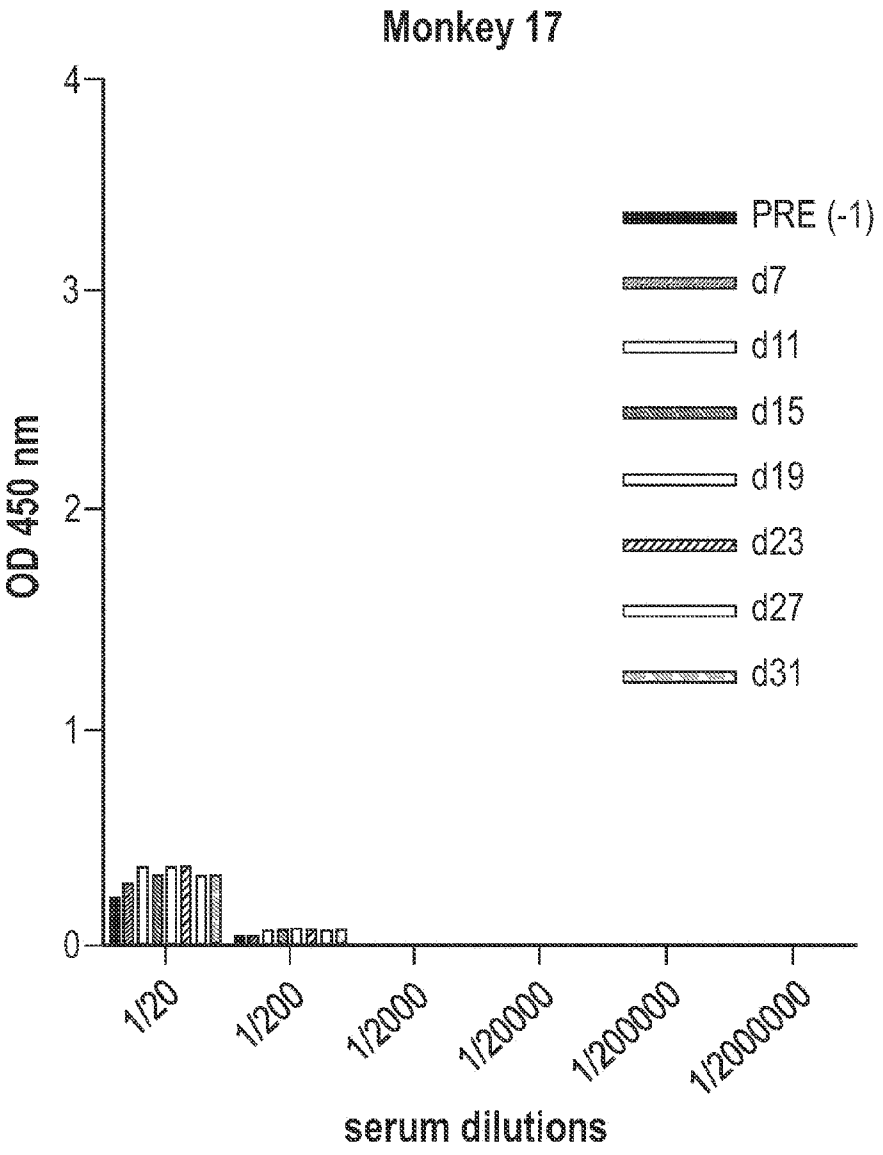
Monkey 17
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7O

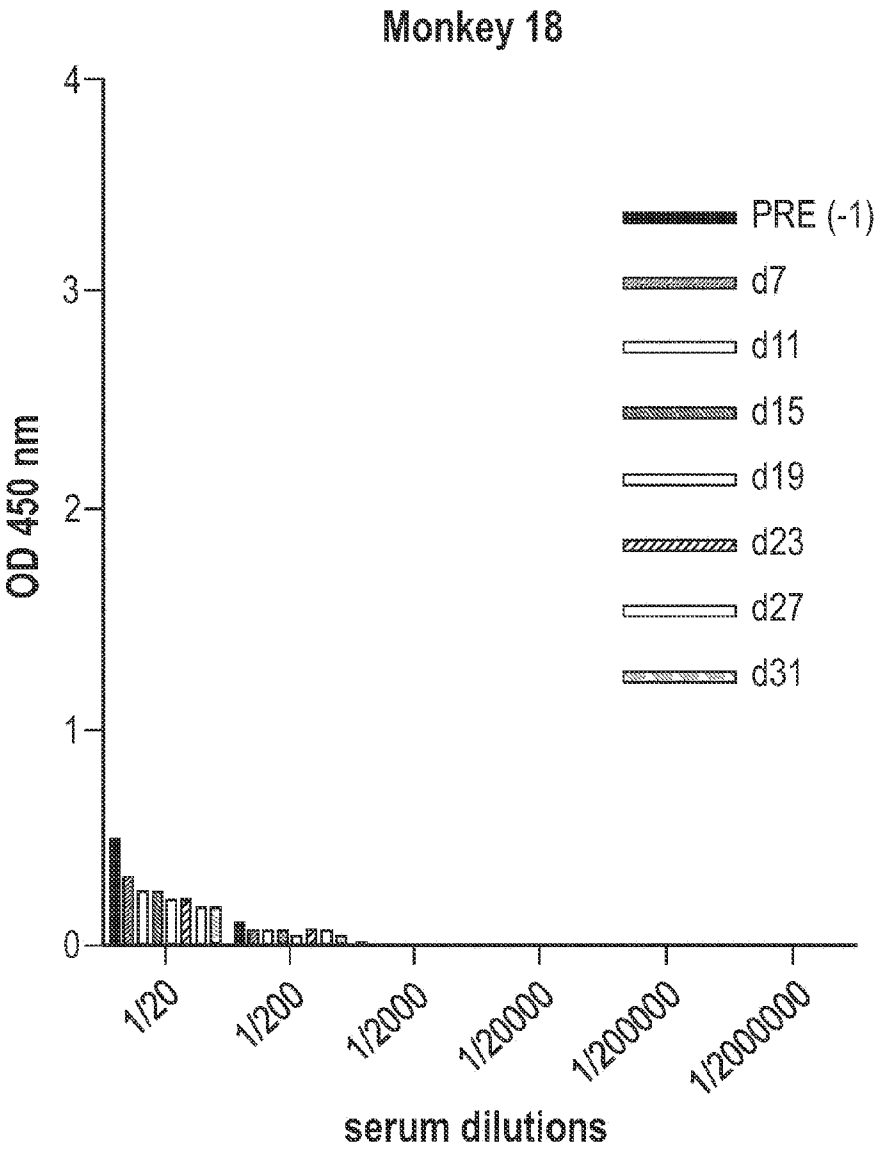
Monkey 18
OD 450 nm
4
3
2
1
0
PRE (-1)
d7
d11
d15
d19
d23
d27
d31
1/20
1/200
1/2000
1/20000
1/200000
1/2000000
serum dilutions

Fig. 7P

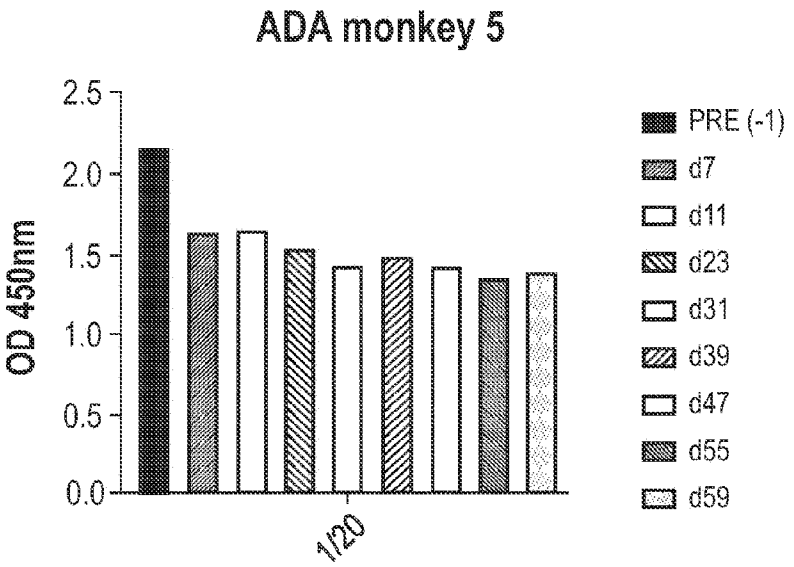
ADA monkey 5
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8A

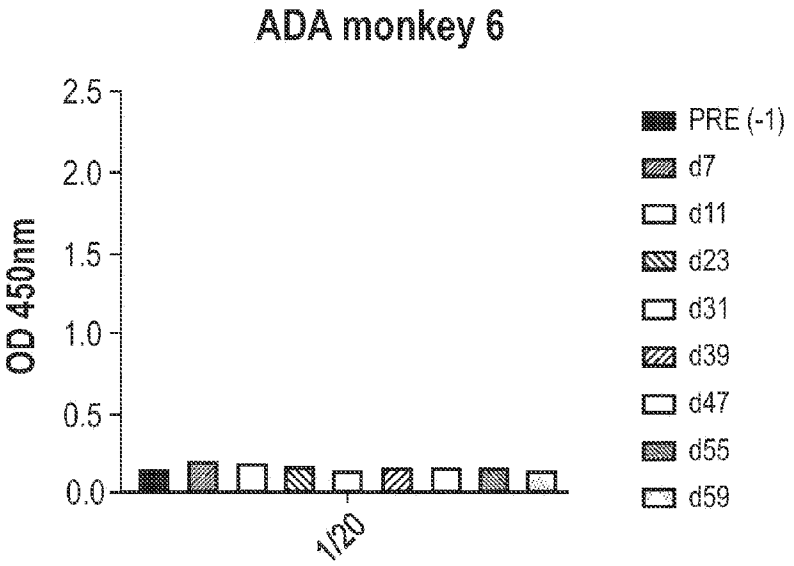
ADA monkey 6
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8B

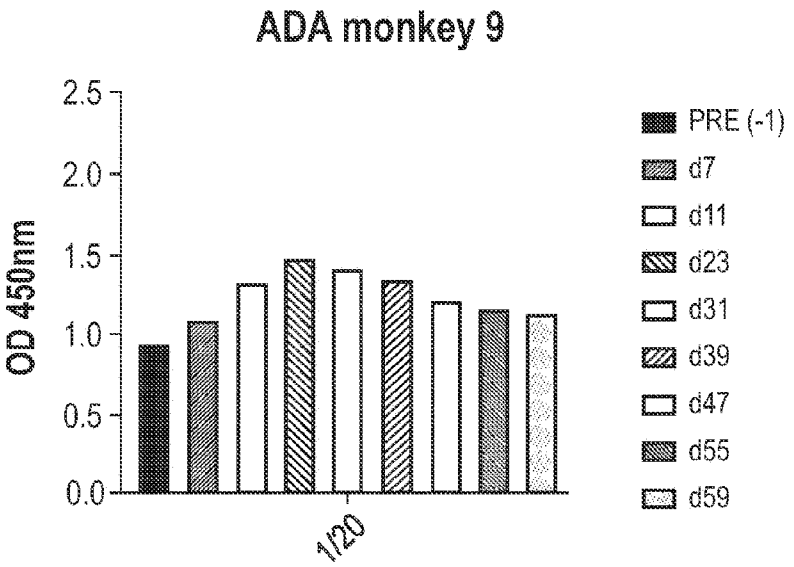
ADA monkey 9
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8C

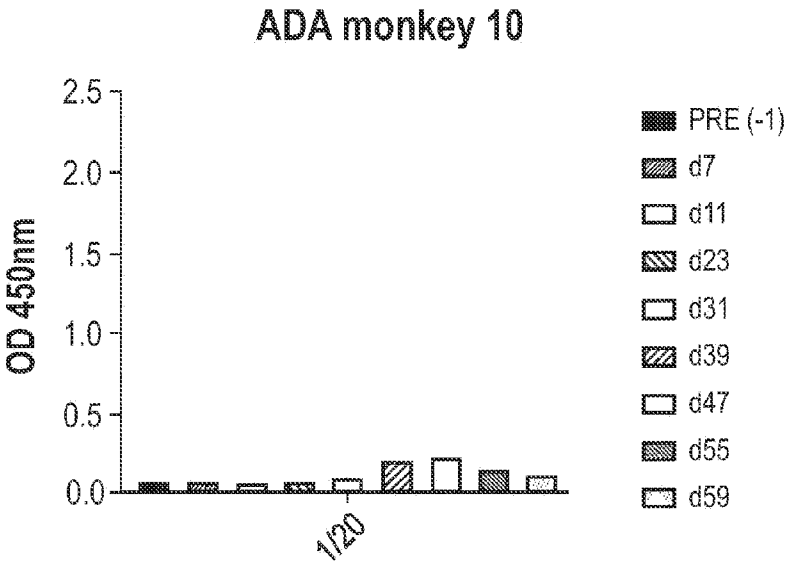
ADA monkey 10
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8D

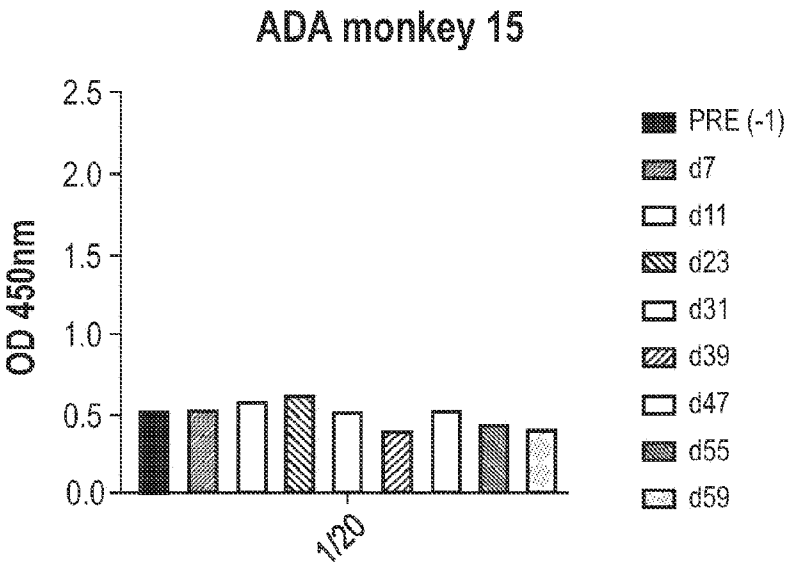
ADA monkey 15
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8E

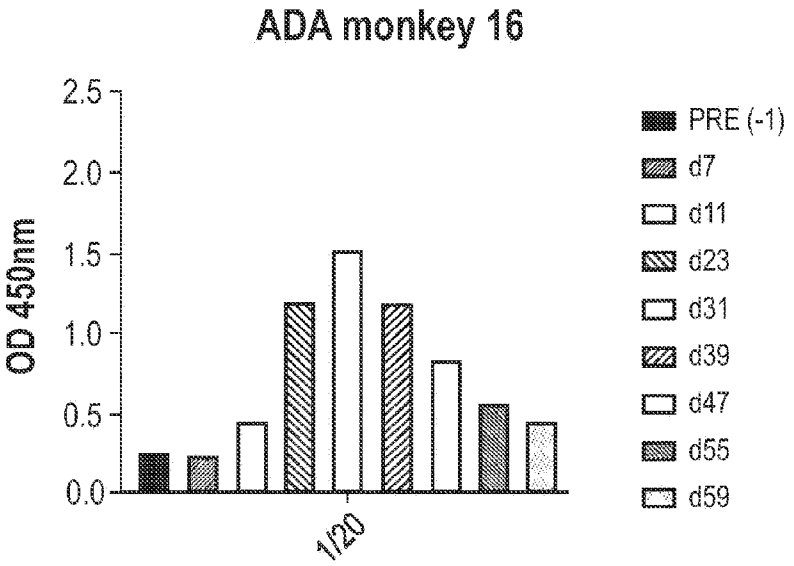
ADA monkey 16
OD 450nm
2.5
2.0
1.5
1.0
0.5
0.0
1/20
PRE (-1)
d7
d11
d23
d31
d39
d47
d55
d59

Fig. 8F

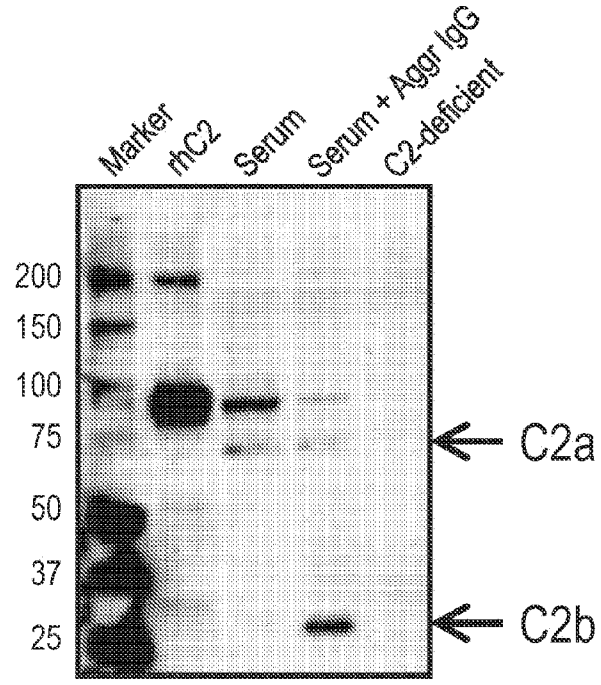
Marker
rhC2
Serum
Serum + Aggr IgG
C2-deficient
200
150
100
75
50
37
25
C2a
C2b

Fig. 9A

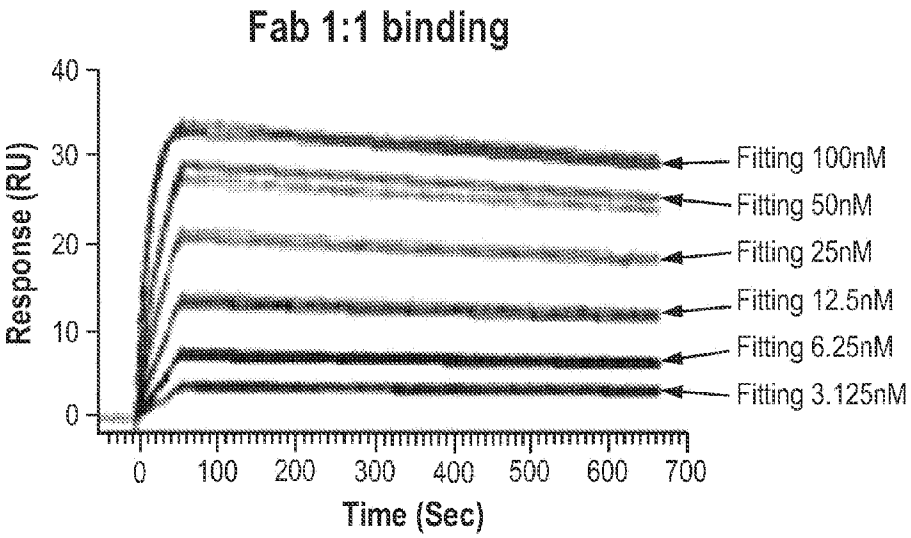
Fab 1:1 binding
Response (RU)
40
30
20
10
0
0
100
200
300
400
500
600
700
Time (Sec)
Fitting 100nM
Fitting 50nM
Fitting 25nM
Fitting 12.5nM
Fitting 6.25nM
Fitting 3.125nM

Fig. 9B

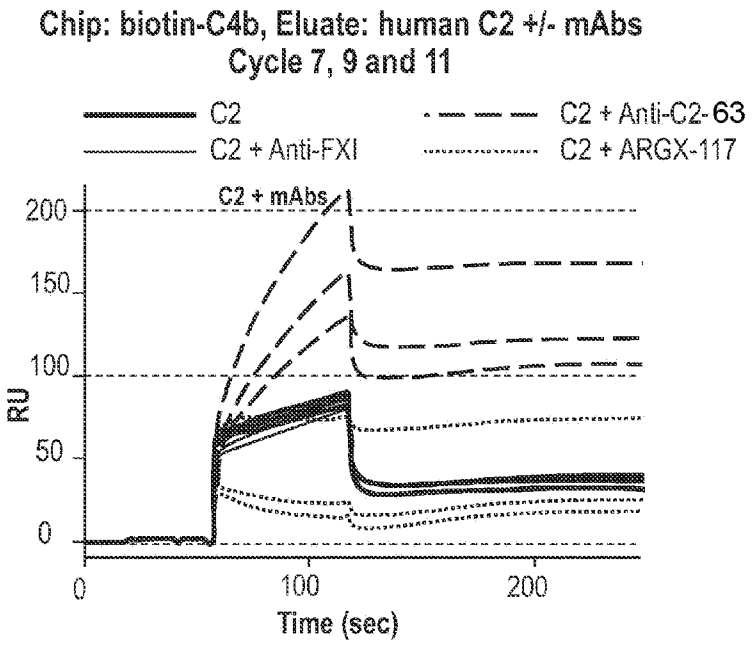
Chip: biotin-C4b, Eluate: human C2 +/- mAbs
Cycle 7, 9 and 11
C2
C2 + Anti-FXI
C2 + Anti-C2-63
C2 + ARGX-117
C2 + mAbs
200
150
100
50
0
RU
0
100
200
Time (sec)

Fig. 9C

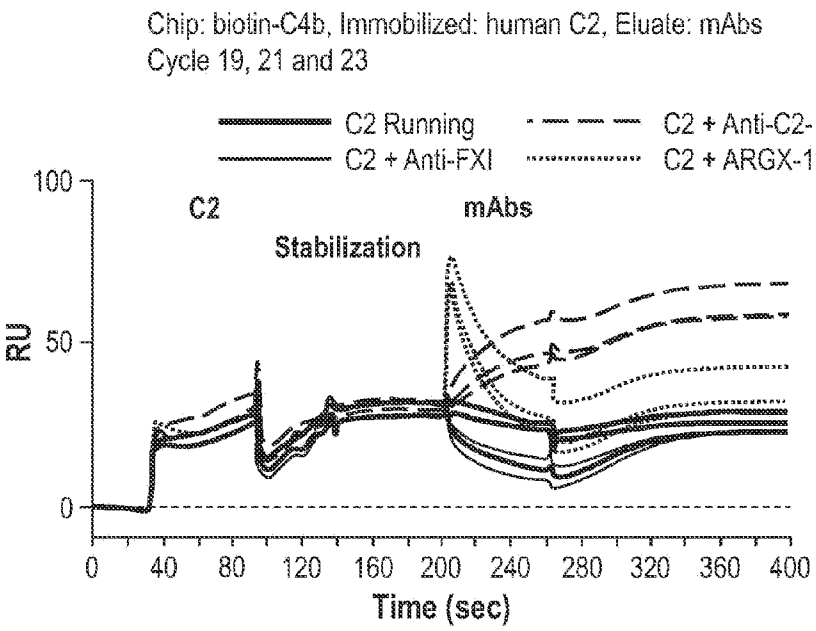
Chip: biotin-C4b, Immobilized: human C2, Eluate: mAbs
Cycle 19, 21 and 23
C2 Running
C2 + Anti-FXI
C2 + Anti-C2-63
C2 + ARGX-117
C2
mAbs
Stabilization
100
50
0
RU
0
40
80
120
160
200
240
280
320
360
400
Time (sec)

Fig. 9D

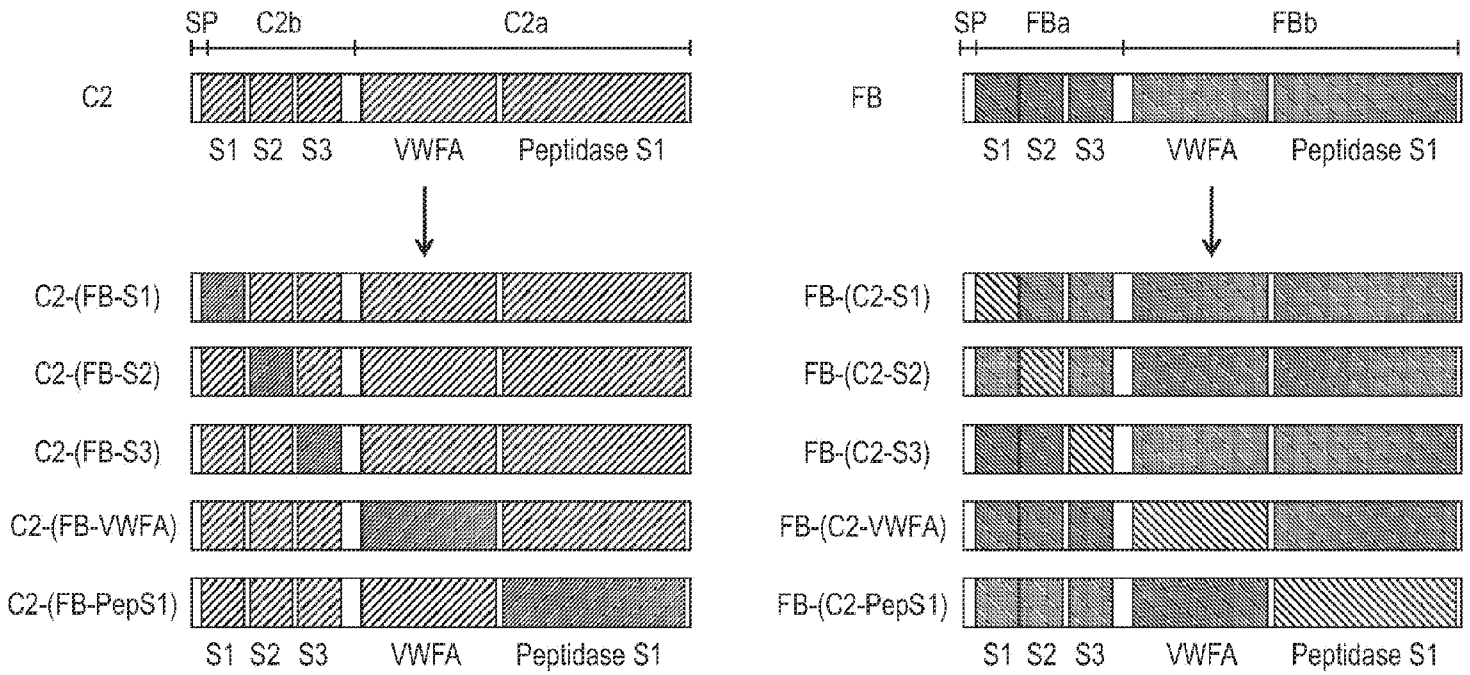

Fig. 10

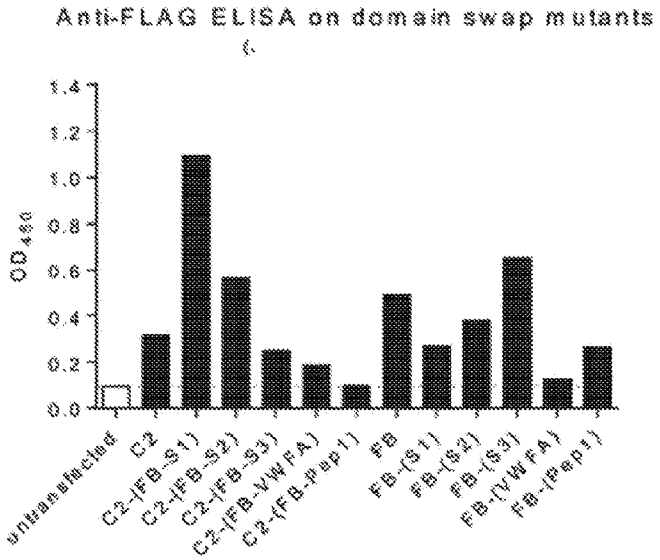
Anti-FLAG ELISA on domain swap mutants
OD450
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
untransfected
C2
C2-(FB-S1)
C2-(FB-S2)
C2-(FB-S3)
C2-(FB-VWFA)
C2-(FB-Pept1)
FB
FB-(S1)
FB-(S2)
FB-(S3)
FB-(VWFA)
FB-(Pept1)

Fig. 11

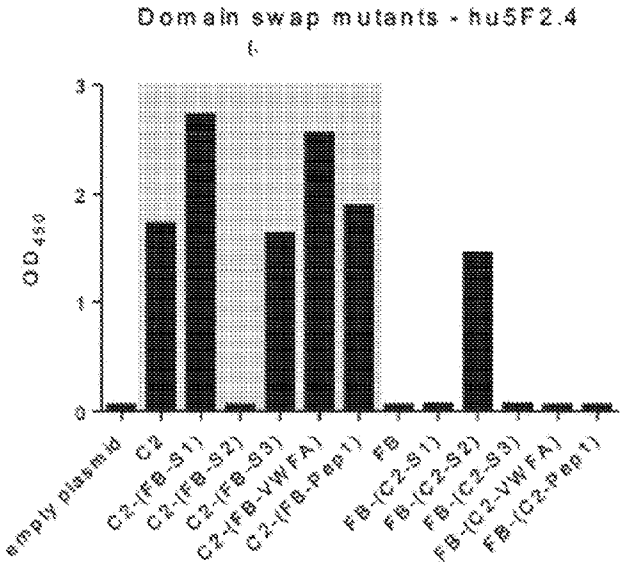
Domain swap mutants - hu5F2.4
OD450
0
1
2
3
empty plasmid
C2
C2-(FB-S1)
C2-(FB-S2)
C2-(FB-S3)
C2-(FB-VWFA)
C2-(FB-Pept)
FB
FB-(C2-S1)
FB-(C2-S2)
FB-(C2-S3)
FB-(C2-VWFA)
FB-(C2-Pept)

Fig. 12

```
Human S2      VRCPAPVSFENGIYTPRLGSYPVGGNVSFECEDGFILRGSPVRQCRPNGMWDGETAVCDNG
Mouse S2      VRCLAPSSFENGIYFPRLVSYPVGSNVSFECEQDFTLRGSPVRYCRPNGLWDGETAVCDNG
              *** ** ******* *** ***** *******  * ******* ***** ***********
```

Fig. 13

DK/EP 3893924 T3

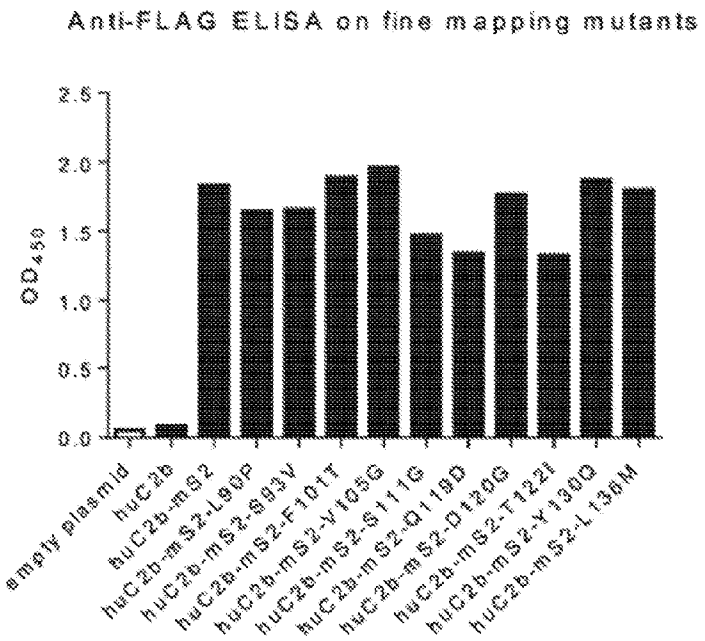
Anti-FLAG ELISA on fine mapping mutants
OD450
2.5
2.0
1.5
1.0
0.5
0.0
empty plasmid
huC2b
huC2b-mS2
huC2b-mS2-L99P
huC2b-mS2-S93V
huC2b-mS2-F101T
huC2b-mS2-V105G
huC2b-mS2-S111G
huC2b-mS2-Q115D
huC2b-mS2-D125G
huC2b-mS2-T122I
huC2b-mS2-Y135D
huC2b-mS2-L136M

Fig. 14

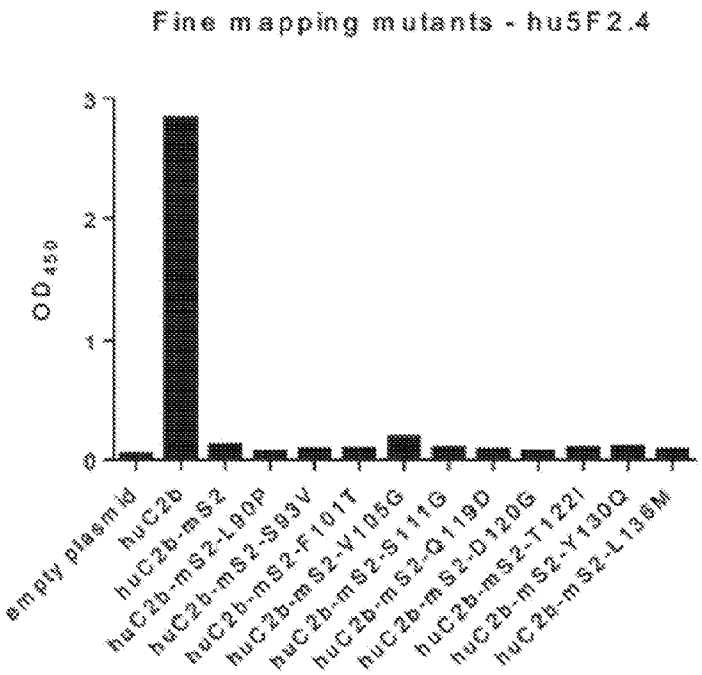
Fine mapping mutants - hu5F2.4
OD450
0
1
2
3
empty plasmid
huC2b
huC2b-mS2
huC2b-mS2-L80P
huC2b-mS2-S93V
huC2b-mS2-F101T
huC2b-mS2-V105G
huC2b-mS2-S111G
huC2b-mS2-Q119D
huC2b-mS2-D120G
huC2b-mS2-T122I
huC2b-mS2-Y130C
huC2b-mS2-L136M

Fig. 15

Cluster 1

```
Human C2      VRCPAPVSFENGIYTPRLGSYPVGGNVSFECEDGFILRGSPVRQCRPNGMWDGETAVCDNG
Mouse C2      VRCLAPSSFENGIYFPRLVSYPVGSNVSFECEQDFTLRGSPVRYCRPNGLWDGETAVCDNG
              *** ** ******* *** ***** *******  * ******* ***** ***********
```

Cluster 2

```
Human C2      VRCPAPVSFENGIYTPRLGSYPVGGNVSFECEDGFILRGSPVRQCRPNGMWDGETAVCDNG
Mouse C2      VRCLAPSSFENGIYFPRLVSYPVGSNVSFECEQDFTLRGSPVRYCRPNGLWDGETAVCDNG
              *** ** ******* *** ***** *******  * ******* ***** ***********
```

Cluster 3

```
Human C2      VRCPAPVSFENGIYTPRLGSYPVGGNVSFECEDGFILRGSPVRQCRPNGMWDGETAVCDNG
Mouse C2      VRCLAPSSFENGIYFPRLVSYPVGSNVSFECEQDFTLRGSPVRYCRPNGLWDGETAVCDNG
              *** ** ******* *** ***** *******  * ******* ***** ***********
```

Fig. 16

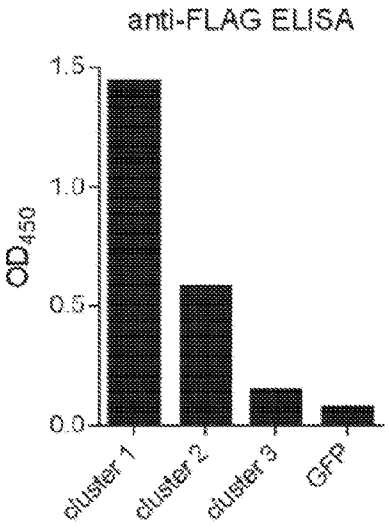

Fig. 17A

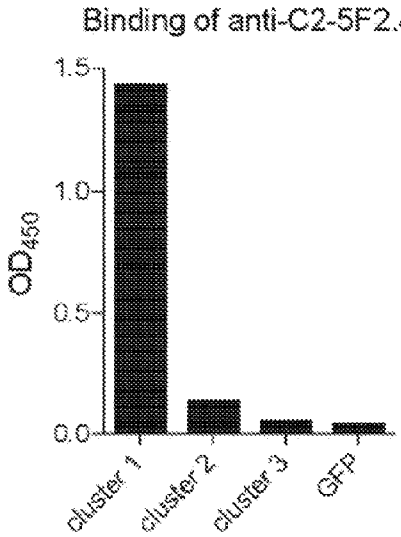

Fig. 17B

SEKVENSLISTE

Sekvenslisten er udeladt af skriftet og kan hentes fra det Europæiske Patent Register.

The Sequence Listing was omitted from the document and can be downloaded from the European Patent Register.