(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
21 July 2022 (21.07.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/155340 A1

(51) International Patent Classification:
C07K 16/28 (2006.01) *A61K 39/395* (2006.01)
A61P 37/00 (2006.01)

(21) International Application Number:
PCT/US2022/012317

(22) International Filing Date:
13 January 2022 (13.01.2022)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
63/137,089 13 January 2021 (13.01.2021) US
63/274,748 02 November 2021 (02.11.2021) US

(71) Applicant: **VISTERRA, INC.** [US/US]; 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US).

(72) Inventors: **VISWANATHAN, Karthik**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US). **BOOTH, Brian**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US). **RAMAKRISHNAN, Boopathy**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US). **WOLLACOTT, Andrew**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US). **BABCOCK, Gregory**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US). **SHRIVER, Zachary**; C/o Visterra, Inc., 275 2nd Avenue, 4th Floor, Waltham, MA 02451 (US).

(74) Agent: **KIM, Sang-A**; Proskauer Rose LLP, One International Place, Boston, MA 02110 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, IT, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW,

(54) Title: HUMANIZED COMPLEMENT 5A RECEPTOR 1 ANTIBODIES AND METHODS OF USE THEREOF

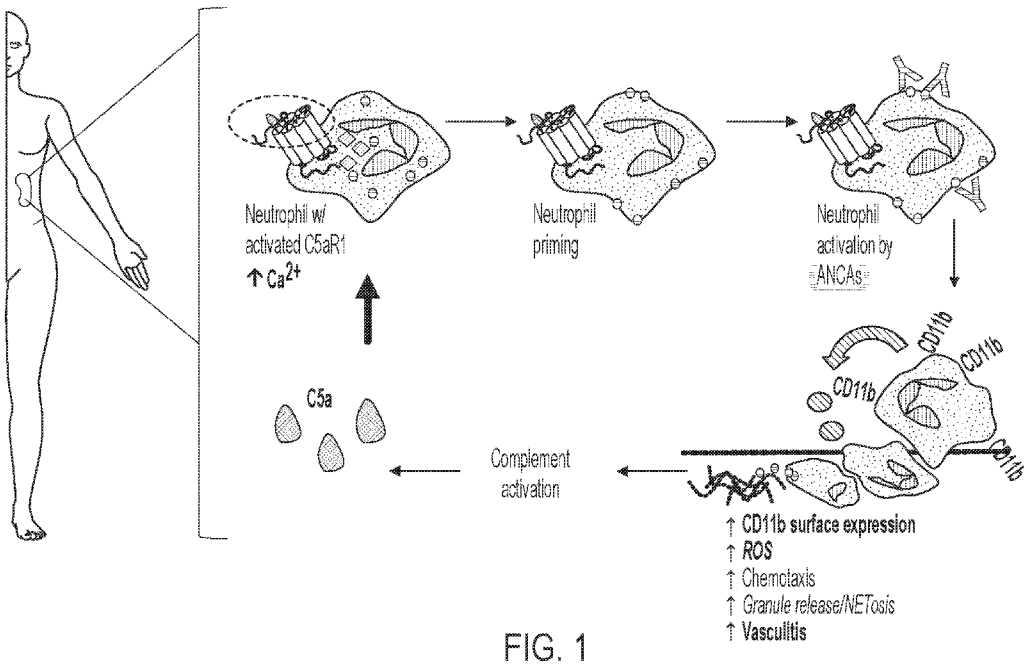


FIG. 1

(57) **Abstract:** The present disclosure provides, among other things, two different formats of humanized antibodies against human complement component 5a receptor 1. The disclosure also provides a method of treating a subject having dysfunctions of C5a/C5aR1 axis pathway, including but not limited to ANCA-associated vasculitis, comprising administering to the subject in need thereof a an effective amount of antibody or a nucleic encoding an antibodies binding to C5aR1 described herein, and wherein administering results in a decrease in symptoms associated with C5a/C5aRl associated dysfunction in the subject.

WO 2022/155340 A1

[Continued on next page]

SA, SC, SD, SE, SG, SK, SL, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, WS, ZA, ZM, ZW.

(84) **Designated States** *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*

HUMANIZED COMPLEMENT 5A RECEPTOR 1 ANTIBODIES AND METHODS OF USE THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to U.S. Provisional Application Number 63/137,089, filed January 13, 2021, and U.S. Provisional Application Number 63/274,748, filed November 02, 2021, the disclosure of each of which is hereby incorporated by reference in its entirety.

BACKGROUND

[0002] Disclosed are compositions and methods for the reduction of auto-immune diseases and disorders associated with complement 5a/complement 5a receptor 1 C5a/C5aR1 mediated immune inflammation. The C5a–C5aR1 axis is of particular interest for therapeutic intervention in order to block attraction of neutrophils to local sites, inhibit neutrophil activation as well as vascular destruction. The compositions and methods disclosed herein, may include a step of administering a C5aR1 antagonist, as well as methods of treating a subject in need of such a treatment.

SUMMARY OF THE INVENTION

[0003] The present disclosure provides, among other things, anti-C5aR1 antibodies with increased specificity to C5aR1 and therapeutic uses of such antibodies in effectively treating diseases or disorders associated with C5 and its receptors, such as, ANCA-vasculitis, typical hemolytic uremic syndrome, age-related macular degeneration, rheumatoid arthritis, sepsis, severe burn, antiphospholipid syndrome, asthma, lupus nephritis, Goodpasture's syndrome, and chronic obstructive pulmonary disease. As described herein, the present disclosure is, in part, based on identification of humanized anti-C5aR1 specific antibodies that bind to certain regions on Site I and/or Site II of C5aR1 and have significantly reduced cross reactivity to C5aR2 or any other G protein-coupled receptors. In particular, anti-C5aR1 antibodies of the present disclosure are characterized with high binding affinity to C5aR1 (e.g., with K_D less than 50 nM) and minimal cross-reactivity with C5aR2. This is significant because C5aR1-antibodies of the

present disclosure allow potent inhibition of C5aR1 signaling in the presence of high C5a concentrations. As a result, C5aR1-antibodies of the present disclosure can be used at a lower dose to achieve therapeutic effect relative to the other anti-C5aR1 antibodies or C5a-antibodies. This is demonstrated by the surprisingly high potency observed in functional assays, relative to prior-art antibodies, as described herein. Moreover, highly potent Site I C5aR1 antibodies of the present disclosure compete with each other for Site I, and highly potent Site II C5aR1 antibodies of the present disclosure compete with each other on Site II. Additionally, the present disclosure provides methods and compositions for inhibiting C5aR1 and/or C5a signaling by targeting both Site I and Site II of C5aR1. Simultaneous targeting of Site I and Site II significantly may enhance inhibitory activity. For example, combination of Site I and Site II antibodies or bispecific antibodies (e.g., biparatopic), but not two Site II or two Site II antibodies, significantly enhance activity. Inventive anti-C5aR1 antibodies of the present disclosure promise a more potent treatment of complement mediated diseases and disorders, particularly ANCA-vasculitis.

[0004] Additionally, the present disclosure provides, among other things, anti-C5aR1-antibodies comprising Fc variants that have significantly reduced ADCC, ADCP and CDC function. As described herein, the anti-C5aR1 antibodies of present disclosure comprise novel combinations of mutations that abolish binding to all FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa, FcγRIIIb, and C1q, and maintain its ability to bind to FcRn.

[0005] In some embodiments, the C5aR1 antibodies provided herein have a wildtype IgG4 Fc domain. In some embodiments, the C5aR1 antibodies provided herein have a modified IgG4 Fc domain. In some embodiments, the modified C5aR1 antibodies comprise a Fab arm exchange mutation. In some embodiments, the modified C5aR1 antibodies further comprise Fc silencing mutations.

[0006] The C5a–C5aR1 axis is of particular interest for therapeutic intervention in order to block attraction of neutrophils to local sites, inhibit neutrophil activation as well as vascular destruction. The compositions and methods disclosed herein, may include a step of administering a C5aR1 antagonist, as well as methods of treating a subject in need of such a treatment.

[0007] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof binding to at least one of sequences of C5aR1, comprising sequences of SEQ ID NO: 1, SEQ ID NO: 2 or SEQ ID NO: 3.

[0008] In some embodiments, an antibody or antigen binding fragment thereof binds to SEQ ID NO: 1 of C5aR1. In some embodiments, an antibody or antigen binding fragment thereof binds to SEQ ID NO: 2 of C5aR1. In some embodiments, an antibody or antigen binding fragment thereof binds to SEQ ID NO: 3 of C5aR1.

[0009] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 14.

[0010] In some embodiments, VH comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 93% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 97% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 14. In some embodiments, VH comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 14.

[0011] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a light chain variable region (VL), wherein the VL comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 25.

[0012] In some embodiments, VL comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 93% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 97% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 25. In some embodiments, VL comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 25.

[0013] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof, comprising a VH region, wherein the VH comprises three heavy chain complementarity determining regions (HCDRs), wherein the HCDR1, HCDR2 and HCDR3 sequences comprising amino acid sequences of SEQ ID Nos: 6 (NYWMH), 7 (YLNPSSGYTKYAQKFQG) and 8 (SGGDNYGNPYYFDR), respectively.

[0014] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof, comprising a VL region, wherein the VL comprises three light chain complementarity determining regions (LCDRs), wherein the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID Nos: 9 (RASQSIVHSNGNTYLH), 10 (KVSNRFS) and 11 (AQYTLVPLT), respectively.

[0015] In one aspect, this disclosure presents an antibody or antigen binding fragment thereof, comprising a VH region, wherein the VH comprises three heavy chain complementarity determining regions (HCDRs), wherein the HCDR1, HCDR2 and HCDR3 sequences comprising amino acid sequences of SEQ ID Nos: 6 (NYWMH), 7 (YLNPSSGYTKYAQKFQG) and 8 (SGGDNYGNPYYFDR), respectively and a VL region, wherein the VL comprises three light chain complementarity determining regions (LCDRs), wherein the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID Nos: 9 (RASQSIVHSNGNTYLH), 10 (KVSNRFS) and 11 (AQYTLVPLT), respectively.

[0016] In one embodiment, the antibody or antibody or antigen binding fragment thereof according to this disclosure, further comprises an Fc region.

[0017] In one embodiment, the antibody or antibody or antigen binding fragment thereof according to this disclosure, further comprises an Fc region, wherein the Fc domain is independently selected from IgG1, IgG2, IgG3, and IgG4.

[0018] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits interaction of complement component 5a (C5a) with C5aR1.

[0019] In one embodiment, the antibody or antigen binding fragment thereof does not bind to C5aR2 or any other GPCR.

[0020] In one embodiment, the antibody or antigen binding fragment thereof is humanized.

[0021] In one embodiment, the VH or the VL of the antibody or the antigen binding fragment thereof, has been modified to enhance the stability of the molecule.

[0022] In one embodiment, the antibody or the antigen binding fragment thereof, comprises a serine or tyrosine mutation at position 96 of SEQ ID NO: 5 or SEQ ID NO: 25.

[0023] In one embodiment, the antibody or antigen binding fragment thereof of does not cross-react with mouse C5aR1.

[0024] In one embodiment, the antibody or antigen binding fragment thereof binds C5aR1 at an affinity of between 10 pM to 50 nM.

[0025] In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1with a dissociation constant (K_D) of less than about 100 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 90 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 80 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 75 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 70 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 65 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 60 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 60 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 55 nM. In some embodiments the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 50 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 45 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 40 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 35 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 30 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 25 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 20 nM. In some embodiments the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 15 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 10 nM. In some embodiments, the antibody or antigen binding fragment thereof binds

C5aR1 with a dissociation constant (K_D) of less than about 8 nM. In some embodiments the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 5 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 3 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 1 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 0.5 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 0.1 nM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 100 pM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 80 pM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 50 pM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 25 pM. In some embodiments, the antibody or antigen binding fragment thereof binds C5aR1 with a dissociation constant (K_D) of less than about 10 pM. In one embodiment, the antibody or antigen binding fragment thereof binds C5aR1 at an affinity of 0.16 nM or lower.

[0026] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis.

[0027] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 0.1 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 0.5 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 1 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 3 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 5 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits

neutrophil chemotaxis in the presence of C5a concentration of at least 7 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 10 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 15 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 20 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 25 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 30 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 40 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 50 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 60 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 70 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 80 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 90 nM. In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 100 nM.

[0028] In one embodiment, the antibody or antigen binding fragment thereof inhibits C5a mediated C5aR1 Gα signaling.

[0029] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1inhibits calcium signaling.

[0030] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits CD11b expression.

[0031] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits neutropenia.

[0032] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits β-arrestin signaling.

[0033] In one embodiment, the antibody or antigen binding fragment thereof binding to C5aR1 inhibits ROS production in neutrophils.

[0034] In one embodiment, the antibody or antigen binding fragment thereof is stable at about 4°C for up-to 1 week with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 1-15°C for up-to 1 week with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 2-10°C for up-to 1 week with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 3-8°C for up-to 2 weeks with one or more freeze thaw cycles.

[0035] In one embodiment, the antibody or antigen binding fragment thereof is stable at about 4°C for up-to 2 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 1-15°C for up-to 2 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 2-10°C for up-to 2 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 3-8°C for up-to 2 weeks with one or more freeze thaw cycles.

[0036] In one embodiment, the antibody or antigen binding fragment thereof is stable at about 4°C for up-to 4 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 1-15°C for up-to 4 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 2-10°C for up-to 4 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 3-8°C for up-to 4 weeks with one or more freeze thaw cycles.

[0037] In one embodiment, the antibody or antigen binding fragment thereof is stable at about 4°C for up-to 8 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 1-15°C for up-to 8 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 2-10°C for up-to 8 weeks with one or more freeze thaw cycles. In some embodiments, the antibody or antigen binding fragment thereof is stable at about 3-8°C for up-to 8 weeks with one or more freeze thaw cycles.

[0038] In one aspect, this disclosure encompasses a nucleic acid encoding any antibody or antigen binding fragment thereof described herein.

[0039] In one aspect, this disclosure encompasses a cell comprising the nucleic acid encoding any antibody or antigen binding fragment thereof described herein.

[0040] In one aspect, this disclosure encompasses a method of making an antibody or antigen binding fragment thereof described herein, the method comprising; culturing a host cell comprising a nucleic acid encoding the antibody or antigen binding fragment thereof, an culturing the cell under conditions that allow production of the antibody or antigen binding fragment thereof.

[0041] In one aspect, this disclosure encompasses a method of treating an autoimmune diseases using an antibody or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof binds complement component 5a receptor 1 (C5aR1), the antibody or antigen binding fragment thereof comprising a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 14 and/or an antibody or antigen binding fragment thereof, that binds human complement component 5a receptor 1 (C5aR1) comprising a light chain variable region (VL), wherein the VL comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 25.

[0042] In one embodiment, the method of treating an autoimmune diseases, encompasses using an antibody or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof binds human complement component 5a receptor 1 (C5aR1), the antibody or antigen binding fragment thereof comprising a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence with at least 90% identity to, the amino acid

sequence of SEQ ID NO: 14 and an antibody or antigen binding fragment thereof, that binds human complement component 5a receptor 1 (C5aR1) comprising a light chain variable region (VL), wherein the VL comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 15.

[0043] In one aspect, this disclosure encompasses a method of treating an autoimmune diseases using an antibody or an antigen binding fragment thereof, wherein the antibody or the antigen binding fragment thereof binds complement component 5a receptor 1 (C5aR1), the antibody or antigen binding fragment thereof comprises three heavy chain complementarity determining regions (HCDRs), wherein the HCDR1, HCDR2 and HCDR3 sequences comprising amino acid sequences of SEQ ID Nos: 6 (NYWMH), 7 (YLNPSSGYTKYAQKFQG) and 8 (SGGDNYGNPYYFDR), respectively and three light chain complementarity determining regions (LCDRs), wherein the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID Nos: 9 (RASQSIVHSNGNTYLH), 10 (KVSNRFS) and 11 (AQYTLVPLT), respectively.

[0044] In one aspect, this disclosure encompasses a method of treating an autoimmune diseases using an antibody or an antigen binding fragment thereof, comprising SEQ ID NO: 4 or an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 4.

[0045] In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 70% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 75% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 78% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 80% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 82% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 87% identity to amino acid sequence of SEQ ID NO: 4. In some

embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 90% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 93% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 95% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 97% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 98% identity to amino acid sequence of SEQ ID NO: 4. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 99% identity to amino acid sequence of SEQ ID NO: 4.

[0046] In one aspect, this disclosure encompasses a method of treating an autoimmune diseases using an antibody or an antigen binding fragment thereof, comprising SEQ ID NO: 5 or an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 5.

[0047] In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 70% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 75% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 78% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 80% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 82% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 87% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 90% identity to amino acid sequence of SEQ ID NO: 5. In some

embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 93% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 95% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 97% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 98% identity to amino acid sequence of SEQ ID NO: 5. In some embodiments, an antibody or an antigen binding fragment thereof comprises an amino acid sequence with at least 99% identity to amino acid sequence of SEQ ID NO: 4.

[0048] In one embodiment, this disclosure encompasses a disease caused by neutropenia caused using an antibody or an antigen binding fragment thereof described herein.

[0049] In one embodiment, the neutropenia is caused by high levels of C5a.

[0050] In one embodiment, the disease is ANCA vasculitis or lupus.

[0051] In one embodiment, the disorder is rheumatoid arthritis.

[0052] In one embodiment, the disorder is a kidney disorder.

[0053] In one aspect, this disclosure encompasses a method of inhibition of C5a signaling using an monoclonal antibody which binds to C5aR1, comprising a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 14 and a light chain variable region (VL), wherein the VL comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 25.

[0054] In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising two pairs of antigen binding domains, wherein a first antigen binding domain comprises a VH1 and a VL1, wherein the VH1 and VL1 bind C5aR1 at SEQ ID NO: 3 and wherein a second antigen binding domain comprises a VH2 and VL2, wherein the VH2 and VL2 bind C5aR1 at SEQ ID NO: 1 or SEQ ID NO: 2.

[0055] In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising two pairs of antigen binding domains, wherein an antigen

binding domain comprises a VH1 and a VL1, wherein the VH1 and VL1 bind C5aR1 at SEQ ID NO: 1 or SEQ ID NO: 2 and wherein a second antigen binding domain comprises aVH2 and a VL2, wherein the VH2 and VL2 bind C5aR1 at SEQ ID NO: 3.

[0056] In one embodiment, the biparatopic antibody or antigen binding fragment thereof comprises a VH1 comprising SEQ ID NO: 14 or at least 90% identical to of SEQ ID NO: 14.

[0057] In one embodiment, the biparatopic antibody or antigen binding fragment thereof comprises a VL, wherein the VL1 comprises an amino acid sequence of SEQ ID NO: 15, or is at least 90% identical to amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 84% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 86% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 94% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 96% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 97% identity to the amino acid sequence of SEQ ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ

ID NO: 15. In some embodiments, the VL1 comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 15.

[0058] In one embodiment, the biparatopic antibody or antigen binding fragment thereof comprises a VH2, wherein the VH2 comprises an amino acid sequence of SEQ ID NO: 16, or is at least 90% identical to of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 86% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 94% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 96% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 97% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 16. In some embodiments, the VH2 comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 16.

[0059] In one embodiment, the biparatopic antibody or antigen binding fragment thereof comprises a VL2, wherein the, VL2 comprises an amino acid sequence of SEQ ID NO: 17, or is at least 90% identical to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 75% identity to the amino acid sequence of

SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 84% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 86% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 94% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 96% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 97% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VH2 comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 17. In some embodiments, the VL2 comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 17.

[0060] In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, binding to SEQ ID NO: 3, comprises a heavy chain of SEQ ID NO: 12, or at least 85% identical to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain

comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 84% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 86% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 94% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 96% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 12. In some embodiments, the heavy chain comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 12.

[0061] In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, binding to SEQ ID NO: 3, comprises a light chain of SEQ ID NO: 13, or at least 85% identical to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 75% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 78% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 80% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 82% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 84% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 85% identity to the amino acid sequence of SEQ ID NO:

13. In some embodiments, the light chain comprises an amino acid sequence with at least 86% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 88% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 90% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 92% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 94% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 95% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 96% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 98% identity to the amino acid sequence of SEQ ID NO: 13. In some embodiments, the light chain comprises an amino acid sequence with at least 99% identity to the amino acid sequence of SEQ ID NO: 13.

[0062] In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 85% identical to the amino acid sequence of SEQ ID NO: 12 and a light chain comprising SEQ ID NO: 13, or at least 85% identical to the amino acid sequence of SEQ ID NO: 13. In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 90% identical to the amino acid sequence of SEQ ID NO: 12 and a light chain comprising SEQ ID NO: 13, or at least 90% identical to the amino acid sequence of SEQ ID NO: 13. In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 92% identical to the amino acid sequence of SEQ ID NO: 12 and a light chain comprising SEQ ID NO: 13, or at least 92% identical to the amino acid sequence of SEQ ID NO: 13. In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 95% identical to the amino acid sequence of SEQ ID NO: 12 and a light chain comprising SEQ ID NO: 13, or at least 95% identical to the amino acid sequence of SEQ ID NO: 13. In one aspect, this disclosure encompasses a biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 99% identical to the amino acid sequence of SEQ ID NO: 12

and a light chain comprising SEQ ID NO: 13, or at least 99% identical to the amino acid sequence of SEQ ID NO: 13.

[0063] In one aspect, the present invention provides, among other things, an anti-C5aR1 biparatopic antibody comprising a heavy chain of SEQ ID NO: 71 and a light chain of SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 85% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 90% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 92% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 95% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 97% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 98% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a heavy chain that is at least 99% identical to SEQ ID NO: 71. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 85% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 90% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 92% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 95% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 97% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 98% identical to SEQ ID NO: 72. In some embodiments, an anti-C5aR1 biparatopic antibody comprises a light chain that is at least 99% identical to SEQ ID NO: 72.

[0064] In one aspect, the present invention provides, among other things, an anti-C5aR1 antibody comprising a heavy chain of SEQ ID NO: 69 and a light chain of SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 85% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 90% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 92% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 85% identical to SEQ ID NO: 69.

In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 95% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 97% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 98% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a heavy chain at is at least 99% identical to SEQ ID NO: 69. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 85% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 90% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 92% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 85% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 95% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 97% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 98% identical to SEQ ID NO: 70. In some embodiments, an anti-C5aR1 antibody comprises a light chain at is at least 99% identical to SEQ ID NO: 70.

[0065] In one aspect, this disclosure encompass a biparatopic antibody or antigen binding fragment thereof, wherein the heavy chain comprises the VH1 linked to an Fc domain.

[0066] In one embodiment, the Fc domain of the biparatopic antibody or antigen binding fragment thereof is further linked to a scFv comprising a VH2 comprising an amino acid sequence of SEQ ID NO: 16, or an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 16 and/or a VL2 comprising an amino acid sequence of SEQ ID NO: 17, or an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 17.

[0067] In one embodiment, the Fc domain is independently selected from IgG1, IgG2, IgG3, and IgG4.

[0068] In one embodiment, the scFv is linked to the Fc domain, via a linker.

[0069] In one embodiment, the linker comprises at-least 5 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37. In one embodiment, the linker comprises at-least 3 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37. In

one embodiment, the linker comprises at-least 4 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37. In one embodiment, the linker comprises at-least 6 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37. In one embodiment, the linker comprises at-least 7 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37.

[0070] In one embodiment, the VH2 and VL2 of the biparatopic antibody comprising SEQ ID NO: 16 and SEQ ID NO: 17 are linked to each other via a linker.

[0071] In one embodiment, the linker comprises 1-10 repeats of SEQ ID NO: 31.

[0072] In one embodiment, the VH2 and VL2 of the biparatopic antibody comprising SEQ ID NO: 16 and SEQ ID NO: 17, or an amino acid sequence 90% identical to SEQ ID NO: 16 and SEQ ID NO: 17, further comprise one or more mutations to improve thermal stability of the biparatopic antibody.

[0073] In one embodiment, the biparatopic antibody the mutation to improve thermal stability of the biparatopic antibody comprises incorporation of Cysteine at position 559 of SEQ ID NO: 12 and at position 630 of SEQ ID NO: 12.

[0074] In one embodiment, the biparatopic antibody or antigen binding fragment thereof binding to C5aR1 inhibits interaction of complement component 5a (C5a) with human C5aR1.

[0075] In one embodiment, the biparatopic antibody or antigen binding fragment thereof does not bind to C5aR2.

[0076] In one embodiment, the biparatopic antibody is humanized.

[0077] In one embodiment, the biparatopic antibody does not cross-react with mouse C5aR1.

[0078] In one embodiment, the biparatopic antibody is stable at 4°C for up-to 2 weeks with one freeze thaw cycle. In some embodiments, the biparatopic antibody or antigen binding fragment thereof is stable at about 1-15°C for up-to 2 weeks with one or more freeze thaw cycles. In some embodiments, the biparatopic antibody or antigen binding fragment thereof is stable at about 2-10°C for up-to 2 weeks with one or more freeze thaw cycles. In some embodiments, the biparatopic antibody or antigen binding fragment thereof is stable at about 3-8°C for up-to 2 weeks with one or more freeze thaw cycles.

[0079] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits neutrophil chemotaxis.

[0080] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits neutrophil chemotaxis in the presence of high C5a concentration.

[0081] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 10nM.

[0082] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits C5a mediated C5aR1 Gα signaling.

[0083] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits β-arrestin signaling.

[0084] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits ROS production in neutrophils.

[0085] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits calcium signaling.

[0086] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits CD11b expression.

[0087] In one embodiment, the biparatopic antibody binding to C5aR1 inhibits neutropenia.

[0088] In one aspect, this disclosure encompasses a nucleic acid encoding the biparatopic antibody described herein.

[0089] In one aspect, this disclosure encompasses a cell comprising the nucleic acid encoding the biparatopic antibody described herein.

[0090] In one aspect, this disclosure encompasses a method of making a biparatopic antibody described herein, the method comprising culturing a host cell comprising a nucleic acid encoding the antibody or antigen binding fragment thereof, an culturing the cell under conditions that allow production of the antibody or antigen binding fragment thereof.

[0091] In one aspect, this disclosure encompasses a method of treating an autoimmune diseases using a biparatopic antibody wherein the antibody binds human complement component

5a receptor 1 (C5aR1), the comprising: a first VH and a first VL, VH1 and VL1, wherein the VH1 comprises SEQ ID NO: 14 or an amino acid sequence with at least 90% identity to SEQ ID NO: 14 and wherein the VL1 comprises SEQ ID NO: 15 or an amino acid sequence with at least with at least 90% identity to SEQ ID NO: 15 and a second VH and a second VL, VH2 and VL2, wherein the VH2 comprises SEQ ID NO: 16 or an amino acid sequence with at least with at least 90% identity to SEQ ID NO: 16 and VL2 comprises SEQ ID NO: 17 or an amino acid sequence with at least with at least 90% identity to SEQ ID NO: 17.

[0092] In one embodiment, this disclosure encompasses a method of treating an autoimmune disease caused wherein the autoimmune disease is caused by neutropenia using an antibody or an antigen binding fragment thereof described herein.

[0093] In one embodiment, the neutropenia is caused by high levels of C5a.

[0094] In one embodiment, the disease is ANCA vasculitis or lupus.

[0095] In one embodiment, the disorder is rheumatoid arthritis.

[0096] In one embodiment, the disorder is a kidney disorder.

[0097] In one embodiment, the disorder is stroke.

[0098] In one embodiment, the monospecific or biparatopic C5aR1 antibody of any of the preceding embodiments, wherein the antibody comprises a modified IgG1, IgG4 or IgG2 constant domains.

[0099] In one embodiment, the antibody the C5aR1 comprises a modified IgG4 Fc domain.

[0100] In one embodiment, the modified IgG4 Fc domain comprises substitutions at positions F234, L235 and/or D265.

[0101] In one embodiment, the IgG4 Fc substitution at position F234 is a hydrophobic amino acid selected from Alanine, Valine, Leucine, Isoleucine, phenylalanine, or tryptophan.

[0102] In one embodiment, the IgG4 Fc substitution at position F234 is a valine.

[0103] In one embodiment, the IgG4 Fc substitution at position L235 is an acidic amino acid.

[0104] In one embodiment, the IgG4 Fc substitution at position L235 is an acidic amino acid selected from glutamate or aspartate.

[0105] In one embodiment, the IgG4 Fc substitution at position L235 is aspartate.

[0106] In one embodiment, the IgG4 Fc substitution at position D265 is a non-polar amino acid.

[0107] In one embodiment, the IgG4 Fc substitution at position D265 is a non-polar amino acid selected from alanine, cysteine, glycine, isoleucine, leucine, methionine, and valine.

[0108] In one embodiment, the IgG4 Fc substitution at position D265 is glycine.

[0109] In one embodiment, the antibody of any of the previous embodiments further comprises a substitution at S228.

[0110] In one embodiment, the substitution at S228 is a proline.

[0111] In one embodiment, the monospecific or biparatopic C5aR1 antibody of any of the preceding embodiments, wherein the antibody comprises a modified IgG4 constant domain comprising combination of F234V, L235E and D265G substitutions.

[0112] In one embodiment, the present disclosure provides a method of reducing or preventing antibody dependent cytotoxicity, antibody dependent phagocytosis and/or Complement Dependent Cytotoxicity, using a monospecific or biparatopic C5aR1 antibody of any of the previous embodiments.

[0113] In one aspect, the present disclosure encompasses an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising: a heavy chain variable region (VH) of SEQ ID NO: 14; a light chain variable region (VL) of SEQ ID NO: 25; and a modified Fc domain comprising substitutions F234V, L235E, and D265G.

[0114] In one aspect, the present disclosure encompasses an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising: a heavy chain variable region (VH) comprising HCDR1 of SEQ ID NO: 6 (NYWMH), a HCDR2 of SEQ ID NO: 7 (YLNPSSGYTKYAQKFQG), and a HCDR3 of SEQ ID NO: 8 (SGGDNYGNPYYFDR); a light chain variable region (VL) comprising LCDR1 of SEQ ID NO: 9 (RASQSIVHSNGNTYLH), LCDR2 of SEQ ID NO: 10 (KVSNRFS), and LCDR3 of SEQ ID NO: 11 (AQYTLVPLT); and a modified Fc domain comprising substitutions F234V, L235E,

and D265G.

[0115] In one aspect, the present disclosure encompasses an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a heavy chain of SEQ ID NO: 69 and a light chain of SEQ ID NO: 70.

[0116] In one aspect, the present disclosure encompasses a biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising: a light chain comprising a LCDR1 of SEQ ID NO: 9 (RASQSIVHSNGNTYLH), a LCDR2 of SEQ ID NO: 10 (KVSNRFS), and a LCDR3 of SEQ ID NO: 21 (AQSTLVPLT); and a heavy chain comprising: a HCDR1 of SEQ ID NO: 6 (NYWMH), a HCDR2 of SEQ ID NO: 7 (YLNPSSGYTKYAQKFQG), and a HCDR3 of SEQ ID NO: 8 (SGGDNYGNPYYFDR); a modified Fc domain comprising substitutions F234V, L235E, and D265G; and an scFv comprising a LCDR4 of SEQ ID NO: 22 (RSSQSLVHSNGNTYLN), a LCDR5 of SEQ ID NO: 23 (KVSNRLS), a LCDR6 of SEQ ID NO: 24 (SQSTHVPYT), a HCDR4 of SEQ ID NO: 18 (AYAMS), a HCDR5 of SEQ ID NO: 19 (SISTGGNTYYADSVKG), and a HCDR6 of SEQ ID NO:20 (GYQRFSGFAY), wherein the scFv is linked to the modified Fc domain.

[0117] In one aspect, the present disclosure encompasses a biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising: a first light chain variable region (VL) of SEQ ID NO: 15; a first heavy chain variable region (VH) of SEQ ID NO: 14; a modified Fc domain comprising substitutions F234V, L235E, and D265G; and an scFv comprising a second light chain variable region (VL) of SEQ ID NO: 17 and a second heavy chain variable region (VH) of SEQ ID NO of SEQ ID NO: 16, wherein the scFv is linked to the modified Fc domain.

[0118] In one aspect, the present disclosure encompasses a biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising a light chain of SEQ ID NO: 72 and a heavy chain of SEQ ID NO:71.

[0119] In one aspect, the present disclosure encompasses a biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising: a first light chain variable region (VL) of SEQ ID NO: 25; a first heavy chain variable region (VH) of SEQ ID NO: 14; and a scFv comprising a second light chain variable region (VL) of SEQ ID NO: 17 and a second heavy chain variable region (VH) of SEQ ID NO of

SEQ ID NO: 16, wherein the scFv is linked to the Fc domain. In one embodiment, the biparatopic antibody or an antigen binding fragment thereof comprises a modified Fc domain comprising substitutions F234V, L235E, and D265G, such that the scFv is linked to the modified Fc domain.

[0120] In one aspect, the present disclosure encompasses an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising: a heavy chain variable region (VH) of SEQ ID NO: 43; and a light chain variable region (VL) of SEQ ID NO: 48. In one embodiment, the antibody further comprises a modified Fc domain comprising substitutions F234V, L235E, and D265G.

[0121] In one aspect, the present disclosure encompasses an antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising: a heavy chain variable region (VH) of SEQ ID NO: 14; and a light chain variable region (VL) of SEQ ID NO: 15. In one embodiment, the antibody, further comprises a modified Fc domain comprising substitutions F234V, L235E, and D265G.

BRIEF DESCRIPTION OF FIGURES

[0122] **FIG. 1** is an exemplary schematic diagram showing the pathogenesis of ANCA-vasculitis

[0123] **FIG. 2** is an exemplary schematic diagram of the two kinds of antibodies described in this disclosure.

[0124] **FIG. 3A** is an exemplary schematic diagram of an exemplary biparatopic antibody comprising a scFv linked to heavy chain Fc domain of a Fab described herein. **FIG. 3B** is a schematic diagram of an exemplary biparatopic antibody comprising a scFv linked to the light chain of a Fab as described herein.

[0125] **FIG. 4A** is an exemplary graph showing binding of an exemplary humanized Site II antibody (c2139) and an exemplary biparatopic antibody (c2137-e1711) to C5aR1 using ELISA, as described in this disclosure. **FIG. 4B** is an exemplary graph showing binding of different batches of an exemplary site II antibody (c2139) and an exemplary biparatopic antibody

(c2137-e1711) in U937-C5aR1 cells. FIG. 4C is a graph showing binding of different batches of an exemplary site II antibody (c2139) and an exemplary biparatopic antibody (c2137-e1711) in human neutrophils cells.

[0126] **FIG. 5** is an exemplary graph showing binding of an exemplary humanized Site II antibody (c2139) and biparatopic antibody (c2137-e1711) to C5aR2 using ELISA, as described in this disclosure.

[0127] **FIG. 6A** is an exemplary graph showing inhibition of G-alpha signaling using GeneBLAzer assay using an exemplary humanized Site II antibody, c2139 and an exemplary biparatopic antibody, c2137-e1711 to C5aR1, as described in this disclosure, in the presence of 10nM C5a. Avacopan is shown as a positive control. **FIG. 6B** is an exemplary graph showing inhibition of G-alpha signaling using GeneBLAzer assay using an exemplary humanized Site II, c2139 antibody and an exemplary biparatopic antibody to C5aR1, c2137-e1711, as described in this disclosure, in the presence of 100nM C5a. Avacopan is shown as a positive control. **FIG. 6C** is an exemplary graph showing inhibition of G-alpha signaling using GeneBLAzer assay using an exemplary humanized Site II antibody, c2139 and an exemplary biparatopic antibody, c2137-e1711 to C5aR1, as described in this disclosure, in the presence of 10nM C5a. An anti-C5aR1 control Ab is shown as a positive control. **FIG. 6D** is an exemplary graph showing inhibition of G-alpha signaling using GeneBLAzer assay using an exemplary humanized Site II, c2139 antibody and an exemplary biparatopic antibody to C5aR1, c2137-e1711, as described in this disclosure, in the presence of 100nM C5a. An anti-C5aR1 control Ab is shown as a positive control.

[0128] **FIG. 7A** is an exemplary graph showing inhibition of calcium signaling using an exemplary humanized Site II antibody, as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody. **FIG. 7B** is an exemplary graph showing inhibition of calcium signaling using an exemplary humanized biparatopic antibody, as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody. **FIG. 7C** is an exemplary graph showing inhibition of calcium signaling using avacopan, a known C5aR1 inhibitor, as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody

[0129] **FIG. 8A** is an exemplary graph showing inhibition of neutrophil chemotaxis using an exemplary humanized Site II antibody, c2139 as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody. **FIG. 8B** is an exemplary graph showing inhibition of neutrophil chemotaxis using a humanized biparatopic antibody, c2137-e1711 as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody. **FIG. 8C** is an exemplary graph showing inhibition of neutrophil chemotaxis using avacopan, a known C5aR1 inhibitor, as described in this disclosure, in the presence of increasing concentrations of C5a and increasing concentrations of antibody

[0130] **FIG. 9A** is an exemplary graph showing inhibition of CD11b expression in neutrophils by an exemplary humanized Site II antibody, c2139 and an exemplary biparatopic antibody, c2137-e1711 as described herein, as compared to avacopan, and in the presence of 100nM C5a and increasing antibody concentrations. **FIG. 9B** is an exemplary graph showing inhibition of CD11b expression in neutrophils by an exemplary humanized Site II antibody, c2139 and an exemplary biparatopic antibody, c2137-e1711 as described herein, as compared to avacopan, and in the presence of 10nM an exemplary humanized Site II antibody and an exemplary biparatopic antibody and increasing concentrations of C5a. **FIG. 9C** is an exemplary graph showing inhibition of CD11b expression in neutrophils by an exemplary humanized Site II antibody, c2139 and an exemplary biparatopic antibody, c2137-e1711 as described herein, as compared to 10nM and 100nM anti-C5aR1 control Ab, and in the presence of 10nM an exemplary humanized Site II antibody and an exemplary biparatopic antibody and increasing concentrations of C5a.

[0131] **FIG. 10A** is an exemplary graph showing the inhibition of beta arrestin recruitment on in the presence of exemplary humanized C5aR1 antibodies c2139 and c2137-e1711, each at a dose of 1nM compared to 10nM Avacopan, in the presence of 1nM C5a. **FIG. 10B** is an exemplary graph showing the inhibition of beta arrestin recruitment on in the presence of exemplary humanized C5aR1 antibodies c2139 and c2137-e1711, each at a dose of 1nM compared to 10nM Avacopan, in the presence of 10nM C5a. **FIG. 10C** is an exemplary graph showing the inhibition of beta arrestin recruitment on in the presence of exemplary humanized C5aR1 antibodies c2139 and c2137-e1711, each at a dose of 1nM compared to 10nM Avacopan, in the presence of 100nM C5a.

[0132] **FIG. 11** is an exemplary graph showing the inhibition of ROS signaling in ANCA(-) and ANCA (+) cells as compared to c2139, c2137-e1711, motavizumab and Avacopan.

[0133] **FIG. 12A** is an exemplary graph showing internalization of exemplary humanized anti-10nM C5aR1 antibodies after 0, 6 and 12 hours in C5aR1-U937 cells, as visualized by amine-conjugated antibodies with a pH sensitive fluorescent dye. **FIG. 12B** is an exemplary graph showing internalization of exemplary humanized anti-10nM C5aR1 antibodies after 0, 6 and 12 hours in U937 cells, as visualized by amine-conjugated antibodies with a pH sensitive fluorescent dye. **FIG. 12C** is an exemplary graph showing internalization of exemplary humanized anti-100nM C5aR1 antibodies after 0, 6 and 12 hours in C5aR1-U937 cells, as visualized by amine-conjugated antibodies with a pH sensitive fluorescent dye. **FIG. 12D** is an exemplary graph showing internalization of exemplary humanized anti-100nM C5aR1 antibodies after 0, 6 and 12 hours in U937 cells, as visualized by amine-conjugated antibodies with a pH sensitive fluorescent dye.

[0134] **FIG. 13A** is an exemplary graph showing binding (cross-reactivity) of exemplary humanized C5aR1 antibodies to squirrel monkeys **FIG. 13B** is an exemplary graph showing binding (cross-reactivity) of exemplary humanized C5aR1 antibodies to dogs.

[0135] **FIG. 14A** is an exemplary schematic diagram of the study design in squirrel monkeys showing time of blood draw and dosing. **FIG. 14B** is an exemplary scatter plot of percent change in neutrophil counts from baseline in vehicle, an exemplary humanized Site II antibody and avacopan. **FIG. 14C** is a bar graph of percent change in neutrophil counts from baseline in vehicle, an exemplary humanized Site II antibody and avacopan.

[0136] **FIG. 15A** is an exemplary schematic diagram of the study design in hC5aR1 mice showing time of blood draw and dosing. **FIG. 15B** is an exemplary scatter plot of percent change in neutrophil counts from baseline in vehicle, humanized Site II antibody and avacopan. **FIG. 15C** is an exemplary bar graph of percent change in neutrophil counts from baseline in vehicle, humanized Site II antibody and avacopan.

[0137] **FIG. 16A** is an exemplary graph showing 21-day PK profiles of exemplary tetravalent (biparatopic antibody) and monospecific antibodies. **FIG. 16B** is an exemplary graph showing 500 hour PK profile of Motavizumab in serum of mice. **FIG. 16C** is an exemplary

graph showing 500 hour PK profile of c2139 in serum of mice. **FIG. 16D** is an exemplary graph showing 500 hour PK profile of c2137-e1711 in serum of mice.

[0138] **FIG. 17A is** an exemplary graph showing affinity curves of a monospecific C5aR1 antibody (c2139-Fcmod) and a C5aR1 biparatopic antibody (c2137-e1711-$F_c$mod). **FIG. 17B** is an exemplary graph showing kinetic parameters of C5aR1 biparatopic antibody (c2137-e1711-$F_c$mod). **FIG. 17C** is an exemplary graph showing binding of monospecific C5aR1 antibody (c2139-Fcmod) and a C5aR1 biparatopic antibody (c2137-e1711-$F_c$mod) to C5aR2.

[0139] **FIGs. 18A-18B** demonstrate increase in internalization of the C5aR1 antibodies. **FIG. 18A** is an exemplary graph showing internalization several hours after dissociation of monospecific antibody, c2139. **FIG. 18B** is an exemplary graph showing increase in internalization of biparatopic antibody, c2137-e1711, during association.

[0140] **FIGs. 19A-19B illustrates** inhibition of Gα signaling in the presence of C5aR1 Fc-modified antibodies. **FIG. 19A** shows Gα signaling in the presence of C5aR1 Fc-modified antibodies in the presence of 10nM C5a. **FIG. 19B** shows Gα signaling in the presence of C5aR1 Fc-modified antibodies in the presence of 100nM C5a.

[0141] **FIGs. 20A-20B** is an exemplary series of graphs illustrating inhibition of Calcium signaling in the presence of C5aR1 Fc-modified antibodies - c2137-e1711-$F_c$mod and c2139-Fcmod in comparison to Avacopan and an anti-C5aR1 control Ab in U937-C5aR1 cells. **FIG. 20A** is a dose response curve showing the exemplary graph showing inhibition of calcium signaling using an exemplary Fc modified humanized Site II antibody, as described in this disclosure, in the presence of increasing concentrations of the antibody and 100 nM C5a. **FIG. 20B** is a percent inhibition graph showing inhibition of calcium signaling using an exemplary Fc modified humanized Site II antibody, as described in this disclosure, in the presence of the antibody and 100nM C5a.

[0142] **FIGs. 21A-21D** show the inhibition of calcium signaling in U937-C5aR1 cells as compared to human neutrophils. **FIG. 21A** shows inhibition of calcium signaling in U937-C5aR1 cells in the presence of 10nM C5a. **FIG. 21B** shows inhibition of calcium signaling in U937-C5aR1 cells in the presence of 100nM C5a. **FIG. 21C** shows inhibition of calcium signaling in human neutrophils in the presence of 10nM C5a. **FIG. 21D** shows inhibition of calcium signaling in human neutrophils in the presence of 100nM C5a.

[0143] **FIG. 22A** summarizes percent saturation and F norm of U937-C5aR1 cells incubated with increasing concentrations of antibody, (c2139-Fcmod and c2137-e1711-Fcmod) and 100nM C5a, after 1 hour incubation. **FIG. 22B** summarizes percent saturation and F norm of U937-C5aR1 cells incubated with increasing concentrations of antibody, (c2139-Fcmod and c2137-e1711-Fcmod) and 100nM C5a, after 3 hour incubation.

[0144] **FIGs. 23A-23B** show inhibition of C5a-meditated β-arrestin signaling by c2137-e1711-$F_c$mod and c2139-Fcmod. **FIG. 23A** shows the inhibitory dose-response of β-arrestin signaling by c2137-e1711-$F_c$mod and c2139-Fcmod. **FIG. 23B** shows the percent inhibition of β-arrestin signaling by c2137-e1711-$F_c$mod and c2139-Fcmod.

[0145] **FIGs. 24A-24D** show the inhibition of chemotaxis in C5aR1-U937 stable cells after treatment with C5aR1 antibodies, c2137-e1711-$F_c$mod and c2139-Fcmod, as compared to Avacopan and anti-C5aR1 control Ab. **FIG. 24A** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM c2139-Fcmod and increasing concentration of C5a. **FIG. 24B** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM C5a c2137-e1711-Fcmod and increasing concentration of C5a. **FIG. 24C-24D** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM an anti-C5aR1 control Ab and Avacopan, respectively, in the presence of c2137-e1711-Fcmod.

[0146] **FIGs. 25A-25B** show the inhibition of CD11b signaling in response to treatment with c2137-e1711-$F_c$mod and c2139-$F_c$mod. **FIG. 25A** shows the inhibition of CD11b signaling in the presence of increasing concentration of C5aR1 antagonistic antibody and 10nM C5a. **FIG. 25B** shows the inhibition of CD11b signaling in the presence of increasing concentration of C5aR1 antagonistic antibody and 100nM C5a.

[0147] **FIGs. 26A-26B** show inhibition of ROS signaling in ANCA(-) and ANCA (+) cells as compared to c2139-$F_c$mod, c2137-e1711-$F_c$mod, motavizumab and Avacopan. **FIG. 26A** shows inhibition of ROS production of increasing concentration of monospecific C5aR1 antibody. **FIG. 26B** shows inhibition of ROS production of increasing concentration of biparatopic C5aR1 antibody.

[0148] **FIG. 27A** is an exemplary schematic diagram of the study design in hC5aR1 mice showing time of blood draw and dosing. **FIG. 27B** is an exemplary scatter plot of percent change

in neutrophil counts from baseline in vehicle, c2139, c2139-Fcmod, c2137-e1711, and c2137-e1711-Fcmod. **FIG. 27C** is an exemplary bar graph of percent change in neutrophil counts from baseline in vehicle, c2139, c2139-Fcmod, c2137-e1711, and c2137-e1711-Fcmod.

[0149] **FIG. 28A** is a pictorial representation of infarct size in mouse brain after treatment with indicated doses of Fc modified antibodies as compared to 1mg/kg PMX53. **FIG. 28B** is a graphical representation of infarct size in mouse brain after treatment with indicated doses of Fc modified antibodies as compared to 1mg/kg PMX53.

[0150] **FIGs. 29A-29B** are graphical representations of pharmacokinetics of Fc modified antibodies. **FIG. 29A** is a graphical representation of the percentage of the Fc modified C5aR1 antibodies in the blood serum for 500 hours. **FIG. 29B** is a graphical representation of mean concentration in μg antibody/ml of serum over 500 hours.

[0151] **FIGs. 30A-30F** are graphical representations of PK and PD studies of the Fc modified C5aR1 antibodies, as compared to MVZ-IgG4. **FIG. 30A** is a graphical representation of a dose response curve of c2139Fcmod in serum for 200 hours. **FIG. 30B** is a graphical representation of a dose response curve of c2137-e1711-Fcmod in serum for 200 hours. **FIG. 30C** is a comparison of c2139Fcmod, c2137-e1711-Fcmod and MVZ-IgG4. **FIG. 30D** is *in silico* graphical representation of c2139 at three different concentrations over a period of 500 hours. **FIG. 30E** is an *in silico* graphical representation of c2137-e1711 at three different concentrations over a period of 500 hours. **FIG. 30F** is *in silico* graphical representation of isotype control antibody over a period of 500 hours at a concentration of 20mg/Kg.

[0152] **FIG. 31** is a graphical representation of percent change in neutrophil counts with exemplary C5aR1 antibodies c2139-Fcmod and c2137-e1711-Fcmod at different dosages in human C5aR1 mice.

[0153] **FIG. 32A-32B** are graphical representations of internalization of exemplary C5aR1 antibodies, c2139-Fcmod and c2137-e1711-Fcmod. **FIG. 32A** shows the fluorescence intensity of c2139-Fcmod and c2137-e1711-Fcmod over 0, 6hr and 24hr in U937 cells. **FIG. 32B** shows the internalization of both c2137-e1711-Fcmod and c2139-Fcmod in live cells as observed by Nikon confocal experiment over a period of 300 min.

DEFINITIONS

[0154] *Antibody*: As used herein, the term "antibody" refers to immunoglobulin molecules and immunologically active portions of immunoglobulin (Ig) molecules, i.e., molecules that contain an antigen binding site that binds (immunoreacts with) an antigen. By "binds" or "immunoreacts with" is meant that the antibody reacts with one or more antigenic determinants of the desired. Antibodies include, antibody fragments. Antibodies also include, but are not limited to, polyclonal, monoclonal, chimeric dAb (domain antibody), single chain, Fab, Fab', F(ab')2 fragments, scFvs, and Fab expression libraries. An antibody may be a whole antibody, or immunoglobulin, or an antibody fragment.

[0155] *Antibody dependent cytotoxicity*: As used herein, the term "antibody-dependent cytotoxicity" or "ADCC" refers to lysis of human target cells by an antibody according to the invention in the presence of effector cells.

[0156] *Fab arm exchange*: The term, "Fab arm exchange" refers to the phenomenon that IgG4 antibodies can exchange 'half-molecules', an activity termed Fab arm exchange herein. Especially in bispecific or biparatopic molecules, this results in functionally monovalent antibodies with unknown specificity and hence, potentially, reduced therapeutic efficacy. Mutations can be introduced in the Fc domain to inhibit the Fab arm exchange. It is known that S228P mutation can prevent IgG4 FAE to undetectable levels both in vitro and in vivo.

[0157] *Fc domain*: As used herein, the term "Fc region" refers to a C-terminal region of an immunoglobulin heavy chain that contains at least a portion of the constant region. The term includes native sequence Fc regions and variant Fc regions. In one embodiment, a human IgG heavy chain Fc region extends from Cys226, or from Pro230, to the carboxyl-terminus of the heavy chain. However, the C-terminal lysine (Lys447) of the Fc region may or may not be present. Unless otherwise specified herein, numbering of amino acid residues in the Fc region or constant region is according to the EU numbering system, also called the EU index, as described in Kabat et al., Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991.

[0158] *Humanized antibody:* The term "humanized antibody" includes non-human (e.g., murine) antibodies that are specific immunoglobulin chains, chimeric immunoglobulins, or fragments thereof that contain minimal non-human (e.g., murine) sequences. Typically,

humanized antibodies are human immunoglobulins in which residues from the complementary determining region (CDR) are replaced by residues from the CDR of a non-human species (e.g., mouse, rat, rabbit, hamster) that have the desired specificity, affinity, and capability (Jones et al., *Nature* 321:522-525, 1986; Riechmann et al., *Nature* 332:323-327, 1988; Verhoeyen et al., *Science* 239:1534-1536, 1988).

[0159] *Increased ADCC*: The term "increased ADCC" is defined as either an increase in the maximum percentage of specific lysis observed within the antibody concentration range tested above, and/or a reduction in the concentration of antibody required to achieve one half of the maximum percentage of specific lysis observed within the antibody concentration range tested above. The increase in ADCC is relative to the ADCC, measured with an acceptable, art-recognized assay.

[0160] *Monoclonal Antibody*: The term "monoclonal antibody" refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally-occurring mutations that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

[0161] *Multispecific antibody:* As used herein, the term "multispecific antibody" refers to binding molecules, antibodies, or antigen-binding fragments thereof that have the ability to specifically bind to two or more different epitopes on the same or different target(s).

[0162] *Biparatopic antibody*: As used herein, the term "biparatopic antibody" refers to a multispecific antibody having the capability of binding 2 different non-overlapping epitopes on the same target antigen molecule.

[0163] K_i *or* K_d: As used herein, the term "K_d", as used herein, refers to the dissociation constant of a particular antibody-antigen interaction as is known in the art, and would apply as a parameter of the binding affinity of a targeting moiety to its cognate ligand for the subject compositions.

[0164] *IC50*: As used herein, the term "IC50" refers to the concentration needed to inhibit half of the maximum biological response of the ligand agonist, and is generally determined by competition binding assays.

[0165] *EC50*: As used herein, the term "EC50" refers to a half maximal effective concentration. The term EC50 refers to the concentration of a drug, antibody or toxicant which induces a response halfway between the baseline and maximum after a specified exposure time. More simply, EC50 can be defined as the concentration required to obtain a 50% of the desired effect

[0166] *C5a*: As used herein, the term "C5a" refers to complement component 5a.

[0167] *C5aR1*: As used herein, the term "C5aR1" refers to complement component 5a receptor 1. In some embodiments, the human C5aR1 comprises SEQ ID NO: 38. In some embodiments, certain amino acids of the human C5aR1 comprising SEQ ID NO: 38 have natural variants, such as (N2D and N279K), shown as lowercase letters in Table 1.

[0168] *Linker:* As used herein, the term "linker" refers to a molecule or group of molecules (such as a monomer or polymer) that connects two molecules and often serves to place the two molecules in a preferred configuration. A number of strategies may be used to covalently link molecules together. These include, but are not limited to polypeptide linkages between N- and C-terminus of proteins or protein domains, linkage via disulfide bonds, and linkage via chemical cross-linking reagents. In one aspect of this embodiment, the linker is a peptide bond, generated by recombinant techniques or peptide synthesis. In some embodiments, the linker may contain amino acid residues that provide flexibility. Thus, the linker peptide may predominantly include the following amino acid residues: Gly, Ser, Ala, or Thr. The linker peptide should have a length that is adequate to link two molecules in such a way that they assume the correct conformation relative to one another so that they retain the desired activity. Suitable lengths for this purpose include at least one and not more than 30 amino acid residues. In one embodiment, the linker is from about 1 to 30 amino acids in length. In another embodiment, the linker is from about 1 to 15 amino acids in length. In addition, the amino acid residues selected for inclusion in the linker peptide should exhibit properties that do not interfere significantly with the activity of the polypeptide.

[0169] *Neutrophil*: As used herein, the term "neutrophil" refers to the major class of white blood cells in peripheral blood. Neutrophils have an important role in engulfing and killing extracellular pathogens.

[0170] *scFv*: As used herein, the term "scFv" refers to a fusion protein of the variable regions of the heavy (VH) and light chains (VL) of immunoglobulins, connected with a short linker peptide of ten to about 25 amino acids.

[0171] *Fab*: As used herein, the term "Fab" refers to an antibody fragment comprising a portion of an intact antibody, comprising the antigen-binding or variable region thereof.

[0172] *Neutropenia*: As used herein, the term "neutropenia" refers to low neutrophil count. For example, in a human subject, neutropenia can range from less than 500 ANC to less than 1500 ANC (absolute neutrophil count). The ANC is measured in cells per microliter of blood). Neutropenia in mice is defined as <10 neutrophils/mm^3 blood.

[0173] *In vitro*: As used herein, the term "*in vitro*" refers to events that occur in an artificial environment, e.g., in a test tube or reaction vessel, in cell culture, etc., rather than within a multi-cellular organism.

[0174] *In vivo*: As used herein, the term "*in vivo*" refers to events that occur within a multi-cellular organism, such as a human and a non-human animal. In the context of cell-based systems, the term may be used to refer to events that occur within a living cell (as opposed to, for example, in vitro systems).

[0175] *Subject*: As used herein, the term "subject" refer to a human or any non-human animal (e.g., mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate). A human includes pre- and post-natal forms. In many embodiments, a subject is a human being. A subject can be a patient, which refers to a human presenting to a medical provider for diagnosis or treatment of a disease. The term "subject" is used herein interchangeably with "individual" or "patient." A subject can be afflicted with or is susceptible to a disease or disorder but may or may not display symptoms of the disease or disorder.

[0176] *Dysfunction*: As used herein, the term "dysfunction" refers to an abnormal function. A dysfunction of a molecule (e.g, a protein) can be caused by an increase or decrease in activity associated with such molecule. A dysfunction of a molecule can be caused by a defect

associated with the molecule itself, or other molecules that interact directly or indirectly with or regulate the molecule.

[0177] *Derivatives*: As used herein, the term "derivatives" when used in connection with antibody, or C5aR1 antibodies, refer to a portion having some of the sequence of an original molecule that retains at least some of the functions and/or properties of the original molecule.

[0178] *Identity*: As used herein, the term "identity" refers a relationship between the sequences of two or more polypeptide molecules or two or more nucleic acid molecules as known in the art, comparing the sequences of these molecules. The relationship determined by doing. In the art, "identity" also means the degree of sequence relatedness between nucleic acid molecules or polypeptides, and in some cases more than one nucleotide sequence or more than one. It may be determined by a match between amino acid sequence strings. "Identity" means between a gap alignment (if any) addressed by a particular mathematical model or computer program (i.e., an "algorithm") and a smaller sequence of two or more sequences. Measure the percent identity match.

[0179] *Similarity* or *Similar:* As used herein, the term "similarity" is used in the art with respect to related concepts, but in contrast to "identity," "similarity", refers to both identity and conservative substitution matches. If two polypeptide sequences have, for example, 10 identical amino acids out of 20 amino acids and the rest are all non-conservative substitutions, the percent identity and percent similarity are both 50%. . In the same example, if there are 5 more conservative substitutions, the percent identity remains 50%, but the percent similarity is 75%. Thus, if there are conservative substitutions, the percent similarity between the two polypeptides is higher than the percent identity between these two polypeptides.

[0180] *Treating*: As used herein, the term "treat," "treatment," or "treating" refers to any method used to partially or completely alleviate, ameliorate, relieve, inhibit, prevent, delay onset of, reduce severity of and/or reduce incidence of one or more symptoms or features of a particular disease, disorder, and/or condition. Treatment may be administered to a subject who does not exhibit signs of a disease and/or exhibits only early signs of the disease for the purpose of decreasing the risk of developing pathology associated with the disease.

[0181] *Vector*: The term "vector" refers to a polynucleotide (usually DNA) used to artificially carry foreign genetic material to another cell where it can be replicated or expressed.

Non-limiting exemplary vectors include plasmids, viral vectors, cosmids, and artificial chromosomes. Such vectors may be derived from a variety of sources, including bacterial and viral sources. A non-limiting exemplary viral source for a plasmid is adeno-associated virus.

[0182] Various aspects of the disclosure are described in detail in the following sections. The use of sections is not meant to limit the disclosure. Each section can apply to any aspect of the disclosure. In this application, the use of "or" means "and/or" unless stated otherwise. As used herein, the singular forms "a", "an", and "the" include both singular and plural referents unless the context clearly dictates otherwise.

DETAILED DESCRIPTION

[0183] The present disclosure describes antibodies, nucleic acids and systems for the manufacture thereof and uses and methods for the treatment of diseases associated with an dysfunctional C5a/C5aR1 axis signaling, in particular autoimmune diseases such as, but not limited to ANCA-associated vasculitis, lupus, rheumatoid arthritis, inflammatory bowel disease, C3 glomerulopathy (C3G), hidradenitis suppurativa (HS), atypical hemolytic uremic syndrome

[0184] Lupus nephritis, IgA nephropathy, mayasthenia gravis, macular degeneration, Alzheimers Disease, Amylotrophic Lateral Sclerosis, Huntington's Disease, neuropathic pain, COVID-19 infection, allergic asthma, chronic obstructive pulmonary disease, bullous pemphigoid, pyoderma gangrenosum and psoriasis.

[0185] ANCA-associated vasculitis is a group of diseases (granulomatosis with polyangiitis, eosinophilic granulomatosis with polyangiitis and microscopic polyangiitis), characterized by destruction and inflammation of small vessels. Antineutrophil cytoplasmic autoantibodies (ANCA) are the cause of ANCA-associated vasculitis. Experimental data in animal models and in vitro experiments demonstrate that primed neutrophils are activated by ANCA, which generates C5a that engages C5a receptors on neutrophils. This attracts and in turn primes more neutrophils for activation by ANCA. The C5a binding to C5aR1 may play a central role in the pathogenesis of ANCA-associated vasculitis. The general schematic of ANCA vasculitis is shown in **FIG. 1**. The standard treatment is immunosuppressive therapy with glucocorticoids; these therapies are associated with substantial short- and long-term toxicity.

Avacopan, a C5aR1 binding antagonist small molecule, was recently accepted by the FDA for use in ANCA-associated vasculitis. However, *in vitro* data suggest that avacopan antagonism of C5a can be overcome by high concentrations of C5a. It is known that C5a concentration at the site of inflammation in active AAV may reach 100 nM. It is known that under such conditions the inhibition of C5aR1 by avacopan can be overcome. There is a need for development of robust inhibitors of the C5a/C5aR1 axis for counteracting disorders associated with dysfunction of this pathway. The present disclosure provides compositions and methods of treatment of C5a/C5aR1 axis dysfunction associated disorders, using a biological C5aR1 antagonists.

[0186] In some aspects, provided herewith are two groups of exemplary antibodies binding and antagonizing C5aR1. A schematic representation of the two groups of antibodies in the instant disclosure is shown in **FIG. 2**.

[0187] The energy of agonist binding to the extracellular domain transmits an allosteric conformational change to the transmembrane and intracellular domains, allowing for G-protein binding and signaling. C5aR1 has two known agonists: C5a and $C5a^{desArg}$. C5a has a short half-life in serum as the C-terminal arginine is quickly cleaved by carboxypeptidase N to form $C5a^{desArg}$, which binds to C5aR1 with reduced affinity and displays biased signaling. $C5a^{desArg}$, unlike C5a, does not signal the β-arrestin pathway and does not stimulate granulocyte release. However, $C5a^{desArg}$ does stimulate neutrophil chemotaxis, so it is of interest to also block $C5a^{desArg}$ binding to prevent neutrophil migration to the site of inflammation. $C5a^{desArg}$ signaling favors chemotaxis and is not prone to desensitization (*e.g.*, neutrophils continue to migrate until reach high concentrations of C5a, not $C5a^{desArg}$). In an embodiment, an orthosteric antagonist that blocks C5a binding (*e.g.*, an antibody molecule as described herein) also inhibits (*e.g.*, blocks) $C5a^{desArg}$ binding. Inhibition of C5a binding is, in some embodiments, needed at the inflammation site while inhibition of $C5a^{desArg}$ is at periphery and can prevent the migration of neutrophils to the inflammation site. Targeting C5aR1 typically leave the membrane attack complex pathway (C5b) untouched.

Design of monospecific C5aR1 antagonists

[0188] In some embodiments, the antibodies presented herein, bind a site defined by SEQ ID NO: 1 or SEQ ID NO: 2, also called "site I." Site I, typically comprises the N-terminal

residues (*e.g.*, N-terminal 37 residues) of C5aR1 and forms a flexible random coil structure, defined by SEQ ID NO: 1 or SEQ ID NO: 2. An antibody molecule that binds to Site I can typically bind to all residues in Site I or a subset thereof. For example, an antibody molecule that binds to Site I makes contact with one or more residues in Site I. In an embodiment, Site I generally comprises a number of sulfated residues (*e.g.*, sulfated tyrosine resides) and a number of Asp residues.

[0189] In some embodiments, the antibodies presented herein, bind a site defined by SEQ ID NO: 3, also called "site II." Site II, typically comprises the extra cellular loop 2 (ECL2) and the transmembrane residues forming vestibule of C5aR1. In an embodiment, an antibody molecule that binds to Site II binds to ECL2, but does not bind, or does not substantially bind, to ECL1 and/or ECL3. In an embodiment, an antibody molecule that binds to Site II binds to ECL2 and ECL1, but does not bind, or does not substantially bind, to ECL3.

[0190] In some embodiments, the antibody molecules described herein are designed to target Site II, defined by amino acids of SEQ ID NO: 3. In some embodiments amino acid encompassing R175 to G189 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E180 – P183 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E180 – P184 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E178 – P183 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, one or more of the residues R35, H101, V176, V177, R178, E179, E180, Y181, F182, P183 P184, K185, L187, D191, S193, H194, E266, P267, S268, F272, L273 and/or K276 of C5aR1 (SEQ ID NO: 38) are important for binding of Site II antibodies described herein. In some embodiments one or more of the residues E180, Y181, F182, and/or P183 of SEQ ID NO: 38 are critical epitopes for binding of Site II antibodies described herein. In one embodiment, the amino acid residue W102 of SEQ ID NO: 38 is critical for binding of Site II antibodies described herein.

[0191] The sequences of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3 are presented in **Table 1**.

Table 1. The amino acid sequences of Site I, Site II and human C5aR1. Variants (N2D and N279K), are shown as lowercase letters.

Site I sequence	MNSFNYTTPDYGHYDDKDTLDLNTPVDKTSNTLRVPD (SEQ ID NO: 1)
Site I sequence	MDSFNYTTPDYGHYDDKDTLDLNTPVDKTSNTLRVPD (SEQ ID NO: 2)
Site II sequence	RVVREEYFPPKVLCGVDYSHDKRRER (SEQ ID NO: 3)
hC5aR1	MnSFNYTTPDYGHYDDKDTLDLNTPVDKTSNTLRVPDILALVIFA VVFLVGVLGNALVVWVTAFEAKRTINAIWFLNLAVADFLSCLALP ILFTSIVQHHHWPFGGAACSILPSLILLNMYASILLLATISADRF LLVFKPIWCQNFRGAGLAWIACAVAWGLALLLTIPSFLYRVVREE YFPPKVLCGVDYSHDKRRERAVAIVRLVLGFLWPLLTLTICYTFI LLRTWSRRATRSTKTLKVVVAVVASFFIFWLPYQVTGIMMSFLEP SSPTFLLLnKLDSLCVSFAYINCCINPIIYVVAGQGFQGRLRKSL PSLLRNVLTEESVVRESKSFTRSTVDTMAQKTQAV (SEQ ID NO: 38)

[0192] In some aspects, exemplary humanized site I (SEQ ID NO: 1 or SEQ ID NO: 2) binding antibodies, presented herein comprise a heavy chain variable region (HCVR or VH) comprising the amino acid sequences of SEQ ID NO: 16 or SEQ ID NO: 39 to SEQ ID NO: 43 or sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto and/or a light chain variable region (LCVR or VL) comprising an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 44 to SEQ ID NO: 49 or a sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto. **Table 2a** summarizes the amino acid sequences of VH and VL of exemplary site I binding antibodies. In some embodiments, the three heavy chain complementarity determining regions, HCDR1, HCDR2, and HCDR3 are found within the HCVR or VH (SEQ ID NO: 16 or SEQ ID NO: 39 to SEQ ID NO: 43). In some embodiments, three light chain complementarity determining regions, LCDR1, LCDR2, and LCDR3 are found within the LCVR or VL (SEQ ID NO: 17 or SEQ ID NO: 44 to SEQ ID NO: 49). It is noted that An exemplary humanized full

length Site I binding antibody can comprise any combination of a VH selected from an amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 39-43 and a VL selected from an amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 44-49.

[0193] It is noted that a humanized full length Site I binding antibody can comprise any combination of a VH selected from any of amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 39-43 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 16 or SEQ ID NO: 39-43 and a VL selected from any of amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 44-49 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 17 or SEQ ID NO: 44-49.

[0194] In some embodiments, an exemplary site I binding antibody, e1711, comprises a heavy chain of SEQ ID NO: 17 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 17. In some embodiments, the exemplary site I binding antibody, e1711 comprises light chain of SEQ ID NO: 16, or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 16.

[0195] In some embodiments, an exemplary site I binding antibody, e1711, comprises a heavy chain of SEQ ID NO: 43 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 43. In some embodiments, the exemplary site I binding antibody, e1711 comprises light chain of SEQ ID NO: 48, or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 48.

[0196] In some embodiments, the exemplary site I binding antibody, e1711 presented herein comprises a e11L variable light chain and e11H variable heavy chain comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 77 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto and/or a light chain comprising an amino acid sequence of SEQ ID NO: 78 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto.

Table 2a. Variable regions of exemplary humanized Site I binding antibodies

Humanized Site I antibodies: Heavy chain variable regions (SEQ ID NO: 16; SEQ ID NO: 39-43)

e1H	QVQLVQSGAEVKKPGASVKVSCAASGFTFNAYAMSWVRQAPGQGLEWMGSIST GGNTYYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCTRGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 39)
e11H	EVQLVESGGGLVQPGGSLRLSCAASGFTFNAYAMSWVRQATGKGLEWVSSIST GGNTYYPGSVKGRFTISRENAKNSLYLQMNSLRAGDTAVYYCTRGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 40)
e14H	EVQLVESGGGLVQPGGSLRLSCAASGFTFNAYAMSWVRQATGKGLEWVSSIST GGNTYYPGSVKGRFTISRENAKNSLYLQMNSLRAGDTAVYYCARGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 41)
e16H	EVQLLESGGGLVQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKGLEWVSSIST GGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 42)
e17H	EVQLVESGGGLIQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKGLEWVSSIST GGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 43)
e1711H	EVQLVESGGGLIQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKCLEWVSSIST GGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRGYQRFSGFA YWGQGTLVTVSS (SEQ ID NO: 16)
Humanized Site I antibodies: Light chain variable regions (SEQ ID NO: 17; SEQ ID NO: 44-49)	
e3L	DIQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLNWYQQKPGKAPKLLI YKVSNRLSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 44)
e5L	DIVMTQTPLSSPVTLGQPASISCRSSQSLVHSNGNTYLNWLQQRPGQPPRLLI YKVSNRLSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 45)

e6L	DVVMTQTPLSSPVTLGQPASISCRSSQSLVHSNGNTYLNWYQQRPGQPPRLLI YKVSNRLSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 46)
e8L	DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNGNTYLNWYLQKPGQSPQLLI YKVSNRLSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 47)
e11L	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSNGNTYLNWYQQKPGQAPRLLI YKVSNRLSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 48)
e20L	EIVLTQSPGTLSLSPGERATLSCRASQSVVHSNGNTYLNWYQQKPGQAPRLLI YKVSNRLSGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCSQSTHVPYTFGQG TKLEIK (SEQ ID NO: 49)
e1711L	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSNGNTYLNWYQQKPGQAPRLLI YKVSNRLSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSTHVPYTFGCG TKLEIK (SEQ ID NO: 17)

Table 2b. Heavy Chain and Light Chain of exemplary humanized Site I binding antibodies

heavy chain of exemplary Site I monospecific antibody e1711	EVQLVESGGGLIQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKGLEWVSSIST GGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCTRGYQRFSGFA YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSW NSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYK CKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCS VMHEALHNHYTQKSLSLSLGK (SEQ ID NO: 77)
light chain of exemplary Site	EIVLTQSPATLSLSPGERATLSCRSSQSLVHSNGNTYLNWYQQKPGQAPRLLI YKVSNRLSGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCSQSTHVPYTFGQG

I monospecific antibody e1711	TKLEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNAL QSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTK SFNRGEC (SEQ ID NO: 78)

[0197] In some aspects, exemplary site II binding antibodies presented herein comprise a HCVR or VH comprising the amino acid sequences of SEQ ID NO; 14 or SEQ ID NO: 50 to SEQ ID NO: 59 or sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto and/or a LCVR or VL comprising an amino acid sequence of SEQ ID NO: 60 to SEQ ID NO: 68 or a sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto. **Table 3** summarizes the amino acid sequences of VH and VL of exemplary site II binding antibodies. In some embodiments, the three heavy chain complementarity determining regions, HCDR1, HCDR2, and HCDR3 are found within the HCVR or VH (SEQ ID NO: 50 to SEQ ID NO: 59). In some embodiments, three light chain complementarity determining regions, LCDR1, LCDR2, and LCDR3 are found within the LCVR or VL (SEQ ID NO: 60 to SEQ ID NO: 68).

[0198] It is noted that a humanized full length Site II binding antibody can comprise any combination of a VH selected from an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 50-59 or has at least 85, 90, 95, 99 or 100% identity with the amino acid sequence of any of SEQ ID NO: 14 or SEQ ID NO: 50-59 and a VL selected from an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 25 or SEQ ID NO: 60-68 or has at least 85, 90, 95, 99 or 100% identity with the amino acid sequence of any of SEQ ID NO: 15 or SEQ ID NO: 25 or SEQ ID NO: 60-68.

Table 3. Variable regions of exemplary humanized Site II binding antibodies

Humanized Site II antibodies: Heavy chain variable regions (SEQ ID NO: 14; SEQ ID NO: 50-59)	
a4H	QVQLVQSGAEVKKPGASVKVSCKASGYSFSSSWINWVRQAPGQGLEWMGR ISAYDGDTRYAQKLQGRVTMTADKSTSTAYMELRSLRSDDTAVYYCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 50)

a9H	QVQLVQSGAEVKKPGASVKVSCKASGYSFSSSWMNWVRQAPGQRLEWMGR ISAGDGDTRYSQKFQGRVTITADKSASTAYMELSSLRSEDTAVYYCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 51)
a16H	QVQLVQSGAEVKKPGSSVKVSCKASGGSFSSSWINWVRQAPGQGLEWMGR ISPGDGDTRYAQKFQGRVTITADKSTSTAYMELSSLRSEDTAVYYCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 52)
a20H	QVQLVQSGAEVKKPGASVKVSCKASGYSFSSSWINWVRQATGQGLEWMGR MSPGDGDTRYAQKFQGRVTMTANKSISTAYMELSSLRSEDTAVYYCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 53)
a26H	EVQLVQSGAEVKKPGESLRISCKASGYSFSSSWMNWVRQMPGKGLEWMGR ISPGDGDTRYSPSFQGHVTISADKSISTAYLQWSSLKASDTAMYFCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 54)
a30H	EVQLVQSGAEVKKPGESLKISCKGSGYSFSSSWINWVRQMPGKGLEWMGR ISPGDGDTRYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCVRFL ITSTRYVMDYWGQGTTVTVSS (SEQ ID NO: 55)
c2H	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWVRQAPGQGLEWMGY LNPSSGYTKYAQKLQGRVTMTADKSTSTAYMELRSLRSDDTAVYYCTRSG GDNYGNPYYFDRWGQGTTVTVSS (SEQ ID NO: 56)
c13H	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWVRQAPGQRLEWMGY LNPSSGYTKYSQKFQGRVTITRDTSASTAYMELSSLRSEDTAVYYCTRSG GDNYGNPYYFDRWGQGTTVTVSS (SEQ ID NO: 57)
c32H	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWIHWVRQATGQGLEWMGY MNPSSGYTKYAQKFQGRVTMTANKSISTAYMELSSLRSEDTAVYYCTRSG GDNYGNPYYFDRWGQGTTVTVSS (SEQ ID NO: 58)
c21H	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWVRQAPGQGLEWMGY LNPSSGYTKYAQKFQGRVTMTRDTSTSTVYMELSSLRSEDTAVYYCTRSG GDNYGNPYYFDRWGQGTTVTVSS (SEQ ID NO: 14)

c40H	EVQLVQSGAEVKKPGESLKISCKGSGYTFTNYWIHWVRQMPGKGLEWMGY INPSSGYTKYSPSFQGQVTISADKSISTAYLQWSSLKASDTAMYYCTRSG GDNYGNPYYFDRWGQGTTVTVSS (SEQ ID NO: 59)
Humanized Site II antibodies: Light chain variable regions (SEQ ID NO: 15; SEQ ID NO: 25; SEQ ID NO: 60-68)	
a1L	DIQMTQSPSSLSASVGDRVTITCRSSQSLVHSNGNTYLHWYQQKPGKAPK LLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTLVP PTFGQGTKLEIK (SEQ ID NO: 60)
a6L	DVVMTQTPLSSPVTLGQPASISCRSSQSLVHSNGNTYLHWYQQRPGQPPR LLIYKVSNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCSQSTLVP PTFGQGTKLEIK (SEQ ID NO: 61)
a15L	EVVMTQSPATLSVSPGERATLSCRSSQSLVHSNGNTYLHWYQQKPGQAPR LLIYKVSNRFSGIPARFSGSGSGTEFTLTISSLQSEDFAVYYCSQSTLVP PTFGQGTKLEIK (SEQ ID NO: 62)
c4L	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKPGKAPK FLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQSTLVP LTFGQGTKLEIK (SEQ ID NO: 63)
c6L	DVVMTQTPLSSPVTLGQPASISCRSSQSLVHSNGNTYLHWYQQRPGQPPR FLIYKVSNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCSQSTLVP LTFGQGTKLEIK (SEQ ID NO: 64)
c8L	DVVMTQSPLSLPVTPGEPASISCRSSQSLVHSNGNTYLHWYLQKPGQSPQ FLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTLVP LTFGQGTKLEIK (SEQ ID NO: 65)
c9L	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSNGNTYLHWFQQRPGQSPR RLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTLVP LTFGQGTKLEIK (SEQ ID NO: 66)

c10L	DVVMTQSPLSLPVTLGQPASISCRSSQSLVHSNGNTYLHWYQQRPGQSPR FLIYKVSNRFSGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCSQSTLVP LTFGQGTKLEIK (SEQ ID NO: 67)
c38L	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKPGKAPK FLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCSQYTLVP LTFGQGTKLEIK (SEQ ID NO: 68)
c37L	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKPGKAPK FLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCAQSTLVP LTFGQGTKLEIK (SEQ ID NO: 15)
c39L	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKPGKAPK FLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCAQYTLVP LTFGQGTKLEIK (SEQ ID NO: 25)

[0199] In some aspects, an exemplary Site II binding antibody comprises a combination of c39L (SEQ ID NO: 14) and c21H (SEQ ID NO: 25) to produce antibody c2139.

[0200] In some embodiments, the exemplary site II binding antibody, c2139 presented herein comprises a c39L variable light chain and c21H variable heavy chain comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto and/or a light chain comprising an amino acid sequence of SEQ ID NO: 5 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto.

[0201] In some embodiments, the exemplary site II binding antibody, c2137 presented herein comprises a c37L variable light chain and c21H variable heavy chain comprises a heavy chain comprising the amino acid sequence of SEQ ID NO: 4 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto and/or a light chain comprising an amino acid sequence of SEQ ID NO: 75 or a sequence having at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identity thereto.

[0202] In some aspects, an exemplary site II (SEQ ID NO: 3) binding antibody, c2139, presented herein comprises a heavy chain comprising a HCVR or VH, wherein the HCVR or VH comprises three heavy chain complementarity determining regions, HCDR1, HCDR2, and HCDR3, comprising SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8 respectively. In some embodiments, the exemplary site II binding antibody presented herein further comprise a LCVR or VL, wherein the LCVR or VL comprises three light chain complementarity determining regions, LCDR1, LCDR2, and LCDR3, comprising SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11 respectively or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID NOs: 6, 7, 8, 9, 10 and/or 11. The sequences of heavy chain, light chain and HCDR and LCDR are presented in **Table 4a**.

[0203] In some aspects, the exemplary site II binding antibody, c2139, presented herein comprise a HCVR or VH comprising the amino acid sequence of SEQ ID NO: 14 or a sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto and/or a LCVR or VL comprising an amino acid sequence of SEQ ID NO: 25 or a sequence having at least 80%, 85%, 90%, 95% or 99% identity thereto.

[0204] In one aspect, described herein is an exemplary antibody molecule capable of binding to complement component 5a receptor 1 (C5aR1), c2139, wherein the antibody molecule competes with a C5aR1 antibody molecule capable of binding to one or more residues of amino acids of Site II (SEQ ID NO: 3).

[0205] In some embodiments, the exemplary site II binding antibodies comprise a glycine or alanine at position 89 of the VH. In some embodiments the exemplary site II binding antibodies comprise tyrosine at position 91. In some embodiments, the exemplary site II binding antibodies comprise tyrosine at position 91 and glycine or alanine at position 89 of the VH.

Table 4a. The amino acid sequences of heavy, light chains, HCDRs and LCDRs of an exemplary humanized C5aR1 Site II antibodies

Heavy chain of c2139	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWV RQAPGQGLEWMGYLNPSSGYTKYAQKFQGRVTMTRDT STSTVYMELSSLRSEDTAVYYCTRSGGDNYGNPYYFD RWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE

	SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL SLSLGK (SEQ ID NO: 4)
Light chain of c2139	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTY LHWYQQKPGKAPKFLIYKVSNRFSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCAQYTLVPLTFGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 5)
HCDR1 of c2139	NYWMH (SEQ ID NO: 6)
HCDR2 of c2139	YLNPSSGYTKYAQKFQG (SEQ ID NO: 7)
HCDR3 of c2139	SGGDNYGNPYYFDR (SEQ ID NO: 8)
LCDR1 of c2139	RASQSIVHSNGNTYLH (SEQ ID NO: 9)
LCDR2 of c2139	KVSNRFS (SEQ ID NO: 10)
LCDR3 of c2139	AQYTLVPLT (SEQ ID NO: 11)
Heavy chain of c2137	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWV RQAPGQGLEWMGYLNPSSGYTKYAQKFQGRVTMTRDT STSTVYMELSSLRSEDTAVYYCTRSGGDNYGNPYYFD RWGQGTTVTVSSASTKGPSVFPLAPCSRSTSESTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVE SKYGPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPRE

	EQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTC LVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL SLSLGK (SEQ ID NO: 4)
Light chain of c2137	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTY LHWYQQKPGKAPKFLIYKVSNRFSGVPSRFSGSGSGT DFTLTISSLQPEDFATYYCAQSTLVPLTFGQGTKLEI KRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPRE AKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLT LSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 75)

[0206] In some embodiments, the HCVR and LCVR of exemplary Site II binding antibodies, such as for example c2139, are further linked to an Fc domain. The Fc domain further comprises a CH2 and CH3 domain, linked to each other via a linker. However, instant disclosure also encompasses site II binding biologic molecules, devoid of an Fc domain.

[0207] In some embodiments, the exemplary site I or site II binding antibody against C5aR1, binds C5aR1 with a dissociation constant (Kd) of 10 pM to 50 nM, thereby inhibiting the association of C5aR1 with C5a, thereby antagonizing the C5a/C5aR1 axis pathway.

[0208] In some embodiments, the effective inhibition of the C5a/C5aR1 antibody is measured by inhibition of Gα signaling, inhibition of neutrophil chemotaxis, inhibition of CD11b expression and inhibition of calcium signaling.

[0209] In some embodiments, the exemplary site I or site II binding antibody against C5aR1, does not, or does not substantially bind to C5aR2, or other GPCRs.

[0210] In some embodiments, the exemplary site I or site II binding antibody against C5aR1 is capable of binding to human neutrophils.

[0211] In some embodiments, the exemplary site I or site II binding antibody against C5aR1 inhibits β-arrestin signaling.

[0212] In some embodiments, the exemplary site I or site II binding antibody against C5aR1 inhibits ROS production in neutrophils.

[0213] In some embodiments, the exemplary site I or site II binding antibody against C5aR1 is internalized. In some embodiments, internalization takes at least 6 hours. In some embodiments, the internalization takes at least 12 hours. In some embodiments, internalization takes less than 6 hours.

Design of multispecific C5aR1 antagonists

[0214] In some aspects the antibodies presented herein are multispecific antibodies. In an embodiment, the multispecific antibody molecule is a multiparatopic antibody molecule, *e.g.*, it comprises a plurality of immunoglobulin variable region sequences, wherein a first immunoglobulin variable region sequence of the plurality has binding specificity for a first epitope and a second immunoglobulin variable region sequence of the plurality has binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). A bispecific or biparatopic antibody has specificity for no more than two antigens or epitopes. A bispecific or biparatopic antibody molecule is typically characterized by a first immunoglobulin variable region sequence which has binding specificity for a first epitope and a second immunoglobulin variable region sequence that has binding specificity for a second epitope. In an embodiment, a bispecific or biparatopic antibody molecule comprises a half antibody, or fragment thereof, having binding specificity for a first epitope and a half antibody, or fragment thereof, having binding specificity for a second epitope. In an embodiment, the first and second epitopes are on the same antigen, *e.g.*, the same protein (or subunit of a multimeric protein). In an embodiment the first epitope is located on C5aR1 (e.g., Site I, e.g., comprising an N-terminal region as described herein) and the second epitope is located on C5aR1 (e.g., Site II, e.g., comprising an ECL2, as described herein). In an embodiment, the antibody is a biparatopic antibody that binds to Site I (SEQ ID NO: 1 or SEQ ID NO: 2) and Site II (SEQ ID NO: 3) of C5aR1.

[0215] In some aspects, the biparatopic antibody comprises a pair of VH and VL from Table 2a. In some embodiments, the biparatopic antibody comprises a pair of VH and VL from Table 3. In some embodiments, the biparatopic antibody comprises a pair of VH and VL from Table 2a and a pair of VH and VL from Table 3. In some aspects, the biparatopic antibody

comprises a VH selected from an amino acid sequence of SEQ ID NO: 14 or SEQ ID NO: 50-59 or has at least 85%, 90%, 95%, 99% or 100% identity with the amino acid sequence of any of SEQ ID NO: 14 or SEQ ID NO: 50-59 and a VL selected from an amino acid sequence of SEQ ID NO: 15 or SEQ ID NO: 25 or SEQ ID NO: 60-68 or has at least 85%, 90%, 95%, 99% or 100% identity with the amino acid sequence of any of SEQ ID NO: 15 or SEQ ID NO: 25 or SEQ ID NO: 60-68. In some aspects, the biparatopic antibody comprises a VH selected from any of amino acid sequence of SEQ ID NO: 16 or SEQ ID NO: 39-43 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 16 or SEQ ID NO: 39-43 and a VL selected from any of amino acid sequence of SEQ ID NO: 17 or SEQ ID NO: 44-49 or an amino acid sequence 80%, 85%, 90%, 95%, 98%, 99% identical to SEQ ID NO: 17 or SEQ ID NO: 44-49.

[0216] In some embodiments, one epitope of the biparatopic antibody described herein are designed to target sulfated N-terminal peptide or Site I of C5aR1, defined by SEQ ID NO: 1 or SEQ ID NO: 2. In some embodiments, the Site I residues targeted by the antibody molecules described herein, are sulfated. In some embodiments one or more of amino acid residues T8 (threonine 8), D10 (aspartate 10), Y11 (tyrosine 11), Y14 (tyrosine 14) and/or D15 (aspartate 15) are critical Site I epitope contact points. In some embodiments, the sulfation at Y11 and/or Y14 are critical for binding of Site I antibody molecules described herein. In some embodiments, core epitope spans 12 amino acids from T7 to D18 of SEQ ID NO: 38, for binding of Site I antibody molecules described herein. In some embodiments, core epitope spans amino acids from T8 to D18 of SEQ ID NO: 38, for binding of Site I antibody molecules described herein.

[0217] In some embodiments, the antibody molecules described herein are designed to target Site II, defined by amino acids of SEQ ID NO: 3. In some embodiments amino acid encompassing R175 to G189 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E180 – P183 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E180 – P184 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, amino acid encompassing E178 – P183 of SEQ ID NO: 38 are core epitopes for binding of Site II antibody molecules described herein. In some embodiments, one or more of the residues R35, H101, V176, V177, R178, E179, E180, Y181, F182, P183 P184, K185, L187, D191, S193, H194,

E266, P267, S268, F272, L273 and/or K276 of C5aR1 (SEQ ID NO: 38) are important for binding of Site II antibodies described herein. In some embodiments one or more of the residues E180, Y181, F182, and/or P183 of SEQ ID NO: 38 are critical epitopes for binding of Site II antibodies described herein. In one embodiment, the amino acid residue W102 of SEQ ID NO: 38 is critical for binding of Site II antibodies described herein.

[0218] In some embodiments, the biparatopic antibody presented in this disclosure comprises a Fab-Fc and a single chain variable fragment (scFv), wherein the Fc is linked to the scFv via a linker. In some embodiments, the Fab domain binds site I and the scFv binds site II.

[0219] In some embodiments, the biparatopic antibody presented in this disclosure comprises a Fab-Fc and a single chain variable fragment (scFv). In some embodiments, the Fab domain binds site II and the scFv binds site I, wherein the Fc is linked to the scFv via a linker.

[0220] In some embodiments, the biparatopic antibody presented in this disclosure is a tetravalent antibody comprising a heavy chain, wherein the heavy chain comprises a VH-Fc linked to scFV domain, as shown in **FIG. 3A**.

[0221] In some embodiments, the site II binding arm of the biparatopic antibody comprises a glycine or alanine at position 89 of the VH. In some embodiments the site II binding arm of the biparatopic antibody comprise tyrosine at position 91. In some embodiments, the site II binding arm of the biparatopic antibody comprises tyrosine at position 91 and glycine or alanine at position 89 of the VH.

[0222] In some embodiments, the biparatopic antibody presented in this disclosure is tetravalent antibody comprising a light chain, wherein the light chain comprises a VL linked to scFv domain, as shown in **FIG. 3B**.

[0223] In some embodiments, the biparatopic antibody is a bispecific antibody with two arms single chain Fab-Fc design, comprising “knobs-in-holes” (KiH) mutations in CH3 domain, to assemble two half antibodies (common Fc heterodimer and unique VH–CH and VL–CL domains). In some embodiments, the KiH mutations comprise, a T366Y mutation in one CH3 domain can be used to create a knob while an Y407T mutation in the other CH3 domain to create a hole. In some embodiments, F405A mutation in one CH3 domain can be used to create a knob while a T394W mutation in the other CH3 domain to create a hole. In some embodiments, a

T366W mutation in one CH3 domain can be used to create a knob while an Y407A mutation in the other CH3 domain to create a hole. In some embodiments, the biparatopic scFv-Fc molecules can be produced with knob-hole technology (e.g., including hole mutations: Y349C, T366S, L368A, Y407V; knob mutations: S354C, T366W).

[0224] In some embodiments, the biparatopic antibody comprises antibody formats described in **Table 4b**.

Table 4b. Formats of biparatopic antibodies

(Site II) Fab-IgG-scFv (Site I)
(Site II) Fab-IgG-linker-VL(Site I)-linker-VH (Site I)
(Site II) Fab-IgG-linker-VH(Site I)-linker-VL (Site I)
(Site I) Fab-IgG-scFv (Site II)

[0225] In an embodiment, the biparatopic anti-C5aR1 antibody molecule comprises two heavy chain variable regions and two light chain variable regions. In an embodiment, the anti-C5aR1 antibody molecule comprises a Fab, F(ab')2, Fv, Fd, or a single chain Fv fragment (scFv).

[0226] In some embodiments, the Fc domain used in this application comprises or is derived from an IgG, IgM, IgE, Fc portion. In addition to the KiH mutations described above, the Fc domain comprises S228P mutation. In some embodiments, the S228P enhanced the homogeneity of the antibody. In some embodiments, the Fc domain comprises or is derived from an IgG Fc domain. In some embodiments, the IgG Fc domain is IgG1, IgG2, IgG3 or IgG4 Fc domain. In some embodiments, the Fc domain is derived from or comprises an IgG4 Fc domain. In some embodiments, the Fc domain is derived from or comprises an IgG4 Fc domain with S228P mutation. In some embodiments, the Fc domain is derived from or comprises an IgG1 Fc domain. In some embodiments, the Fc domain is derived from or comprises an IgG1 Fc domain with S228P mutation.

[0227] In some embodiments, the biparatopic antibody comprises two scFv regions linked to each other via a linker, wherein, the first scFv binds site I and second scFv binds site II.

[0228] In some embodiments, the Fab-Fc-linker-scFv biparatopic antibody is a tetravalent antibody comprising a heavy chain sequence of SEQ ID NO: 12 and light chain sequence of SEQ ID NO: 13 or any sequences that are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 12 and SEQ ID NO 13.

[0229] In some embodiments, the Fab-Fc-linker-scFv biparatopic antibody is a tetravalent antibody comprising a heavy chain sequence of SEQ ID NO: 12 and light chain sequence of SEQ ID NO: 76 or any sequences that are at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 99% identical to SEQ ID NO: 12 and SEQ ID NO 76.

[0230] Table 4c presents the sequences of heavy and light chain sequences of the biparatopic antibody.

Table 4c. The amino acid sequences of heavy and light chains of humanized C5aR1 biparatopic antibody

Heavy chain of c2137-e1711	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWVRQAPGQGL EWMGYLNPSSGYTKYAQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCTRSGGDNYGNPYYFDRWGQGTTVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGG GGGSEIVLTQSPATLSLSPGERATLSCRSSQSLVHSNGNTYLNWY QQKPGQAPRLLIYKVSNRLSGIPARFSGSGSGTDFTLTISSLEPE DFAVYYCSQSTHVPYTFGCGTKLEIKGGGGSGGGGSGGGGSGGGG SEVQLVESGGGLIQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKC LEWVSSISTGGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCTRGYQRFSGFAYWGQGTLVTVSS (SEQ ID NO: 12)
Light chain of c2137-e1711	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKP GKAPKFLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCAQSTLVPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA

	SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 13)
Heavy chain of c2139-e1711	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYWMHWVRQAPGQGL EWMGYLNPSSGYTKYAQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCTRSGGDNYGNPYYFDRWGQGTTVTVSSASTKGPSVFPLA PCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKY GPPCPPCPAPEFLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDG SFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGG GGGSEIVLTQSPATLSLSPGERATLSCRSSQSLVHSNGNTYLNWY QQKPGQAPRLLIYKVSNRLSGIPARFSGSGSGTDFTLTISSLEPE DFAVYYCSQSTHVPYTFGCGTKLEIKGGGGSGGGGSGGGGSGGGG SEVQLVESGGGLIQPGGSLRLSCAASGFTFNAYAMSWVRQAPGKC LEWVSSISTGGNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAED TAVYYCTRGYQRFSGFAYWGQGTLVTVSS (SEQ ID NO: 12)
Light chain of c2139-e1711	DVQMTQSPSSLSASVGDRVTITCRASQSIVHSNGNTYLHWYQQKP GKAPKFLIYKVSNRFSGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCAQYTLVPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC (SEQ ID NO: 76)

[0231] In some embodiments, the biparatopic has two variable regions – variable region 1 and variable region 2. Each variable region comprises a variable heavy chain and a variable light chain.

[0232] In an exemplary embodiment, the variable region 1 comprises variable heavy chain 1 (VH1) comprising SEQ ID NO: 14 and variable light chain 1 (VL1) comprising SEQ ID

NO: 15. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH1, comprising amino acid sequence of SEQ ID NO: 14. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL1, comprising amino acid sequence of SEQ ID NO: 15.

[0233] In some exemplary embodiments the variable region 2 comprises a variable heavy chain 2 (VH2) and a variable light chain 2 (VL2), comprising the amino acid sequence of SEQ ID NO: 16 and SEQ ID NO: 17, respectively. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH2, comprising amino acid sequence of SEQ ID NO: 16. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL2, comprising amino acid sequence of SEQ ID NO: 17.

[0234] In some exemplary embodiments, the biparatopic antibody comprising VH1 of SEQ ID NO: 14, VL1 of SEQ ID NO: 15, VH2 of SEQ ID NO: 16 and VL2 of SEQ ID NO: 17 is called c2137-e1711.

[0235] In some exemplary embodiments, the VH1 comprises three HCDRs, HCDR1, HCDR2 and HCDR3, comprising amino acid sequences of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 6, 7 and/or 8.

[0236] In some exemplary embodiments, the VH2 comprises three HCDRs, HCDR4, HCDR5 and HCDR6, comprising amino acid sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 18, 19 and/or 20.

[0237] In some exemplary embodiments, the VL1 comprises three LCDRs, LCDR1, LCDR2 and LCDR3, comprising amino acid sequences of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 21, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 9, 10 and/or 21.

[0238] In some exemplary embodiments, the VL2 comprises three LCDRs, LCDR4, LCDR5 and LCDR6, comprising amino acid sequences of SEQ ID NO: 22, SEQ ID NO: 23 and

SEQ ID NO: 24, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 22, 23 and/or 24. **Table 5** presents the amino acid sequences of VH1, VH2, VL1, VL2, HCDR1, HCDR2, HCDR3, HCDR4, HCDR5, HCDR6, LCDR1, LCDR2, LCDR3, LCDR4, LCDR5 and LCDR6.

[0239] In some exemplary embodiments, the HCDR1, HCDR2, HCDR3, HCDR4, HCDR5, HCDR6, LCDR1, LCDR2, LCDR3, LCDR4, LCDR5 and LCDR6 comprise an amino acid sequence that differs by no more than 5 amino acid residues from, the amino acid sequence of SEQ ID NOs: 16-21.

Table 5. The amino acid sequences of humanized C5aR1 biparatopic antibody (c2137-e1711) variable domains and CDRs

HCDR1	NYWMH (SEQ ID NO: 6)
HCDR2	YLNPSSGYTKYAQKFQG (SEQ ID NO: 7)
HCDR3	SGGDNYGNPYYFDR (SEQ ID NO: 8)
HCDR4	AYAMS (SEQ ID NO: 18)
HCDR5	SISTGGNTYYADSVKG (SEQ ID NO: 19)
HCDR6	GYQRFSGFAY (SEQ ID NO: 20)
LCDR1	RASQSIVHSNGNTYLH (SEQ ID NO: 9)
LCDR2	KVSNRFS (SEQ ID NO: 10)
LCDR3	AQSTLVPLT (SEQ ID NO: 21)
LCDR4	RSSQSLVHSNGNTYLN (SEQ ID NO: 22)
LCDR5	KVSNRLS (SEQ ID NO: 23)
LCDR6	SQSTHVPYT (SEQ ID NO: 24)

[0240] In some exemplary embodiments a variable region of the biparatopic antibody comprises a variable region 1, wherein the variable region 1 comprises a VH1 and a VL1, comprising the amino acid sequence of SEQ ID NO: 16 and SEQ ID NO: 17, respectively. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH1, comprising amino acid sequence of SEQ ID NO: 16. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL1, comprising amino acid sequence of SEQ ID NO: 17.

[0241] In some exemplary embodiments the variable region of the biparatopic antibody comprises a variable region 2, wherein the variable region 2 comprises a VH2 comprising SEQ ID NO: 14 and a VL2 comprising SEQ ID NO: 15. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH2, comprising amino acid sequence of SEQ ID NO: 14. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL2, comprising amino acid sequence of SEQ ID NO: 15.

[0242] In some exemplary embodiments, the VH1 comprises three HCDRs, HCDR1, HCDR2 and HCDR3, comprising amino acid sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 20, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 18, 19 and/or 20.

[0243] In some exemplary embodiments, the VH2 comprises three HCDRs, HCDR4, HCDR5 and HCDR6, comprising amino acid sequences of SEQ ID NO: 6, SEQ ID NO: 7 and SEQ ID NO: 8, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 6, 7 and/or 8.

[0244] In some exemplary embodiments, the VL1 comprises three LCDRs, LCDR1, LCDR2 and LCDR3, comprising amino acid sequences of SEQ ID NO: 22, SEQ ID NO: 23 and SEQ ID NO: 24, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID Nos: 22, 23 and/or 24.

[0245] In some exemplary embodiments, the VL2 comprises three LCDRs, LCDR4, LCDR5 and LCDR6, comprising amino acid sequences of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 21, or comprises a sequence that differs by no more than 5, 4, 3, 2 or 1 amino acids from amino acids of SEQ ID NOs: 9, 10 and/or 21. **Table 5** presents the amino acid sequences of

VH1, VH2, VL1, VL2, HCDR1, HCDR2, HCDR3, HCDR4, HCDR5, HCDR6, LCDR1, LCDR2, LCDR3, LCDR4, LCDR5 and LCDR6.

[0246] In some exemplary embodiments, the HCDR1, HCDR2, HCDR3, HCDR4, HCDR5, HCDR6, LCDR1, LCDR2, LCDR3, LCDR4, LCDR5 and LCDR6 comprise an amino acid sequence that differs by no more than 5 amino acid residues from, the amino acid sequence of SEQ ID NOs: 16-21.

[0247] In some exemplary embodiments a variable region of the biparatopic antibody comprises a variable region 1, wherein the variable region 1 comprises a VH1 and a VL1, comprising the amino acid sequence of SEQ ID NO: 14 and SEQ ID NO: 25, respectively. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH1, comprising amino acid sequence of SEQ ID NO: 14. In some embodiments the variable region 1 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL1, comprising amino acid sequence of SEQ ID NO: 25.

[0248] In some exemplary embodiments the variable region of the biparatopic antibody comprises a variable region 2, wherein the variable region 2 comprises a VH2 and a VL2, comprising the amino acid sequence of SEQ ID NO: 16 and SEQ ID NO: 17, respectively. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VH2, comprising amino acid sequence of SEQ ID NO: 16. In some embodiments the variable region 2 comprises a sequence 85%, 90%, 95%, 96%, 97%, 98%, or 99% identical to sequence of VL2, comprising amino acid sequence of SEQ ID NO: 17.

[0249] In some exemplary embodiments, the biparatopic antibody comprising VH1 of SEQ ID NO: 14, VL1 of SEQ ID NO: 25, VH2 of SEQ ID NO: 16 and VL2 of SEQ ID NO: 17 is called c2139-e1711.

[0250] In some exemplary embodiments, the exemplary biparatopic antibody binding C5aR1 binds one or more of amino acid residues T8 (threonine 8), D10 (aspartate 10), Y11 (tyrosine 11), Y14 (tyrosine 14) and/or D15 (aspartate 15) in SEQ ID NO: 38.

[0251] In some exemplary embodiments the exemplary biparatopic antibody binding C5aR1 binds to amino acid residues R175-G189 in SEQ ID NO: 38.

[0252] In some exemplary embodiments the exemplary biparatopic antibody binding C5aR1 binds to amino acid residues E180-P183 in SEQ ID NO: 38.

[0253] In some exemplary embodiments the exemplary biparatopic antibody binding C5aR1 binds to amino acid residues E180-P184 in SEQ ID NO: 38.

[0254] In some exemplary embodiments the exemplary biparatopic antibody binding C5aR1 binds to amino acid residues E178-P183 in SEQ ID NO: 38.

[0255] In some embodiments, the exemplary biparatopic antibody against C5aR1, inhibits β-arrestin signaling.

[0256] In some embodiments, the exemplary biparatopic antibody against C5aR1 inhibits ROS production in neutrophils.

[0257] In some embodiments, the exemplary biparatopic antibody against C5aR1 is internalized. In some embodiments, internalization takes at least 6 hours. In some embodiments, the internalization takes at least 12 hours. In some embodiments, internalization takes less than 6 hours.

[0258] In some exemplary embodiments, the mono-specific and biparatopic antibodies can be modified or mutated to enhance the thermal stability of the antibody. The thermal stability of the antibodies can be evaluated by determining the aggregation onset temperature. One of the ways to increase antibody stability is to raise the thermal transition midpoint (Tm) as measured by differential scanning calorimetry (DSC). In general, the protein Tm is correlated with its stability and inversely correlated with its susceptibility to unfolding and denaturation in solution and the degradation processes that depend on the tendency of the protein to unfold. A number of studies have found correlation between the ranking of the physical stability of formulations measured as thermal stability by DSC and physical stability measured by other methods (Maa et al. (1996) Int. J. Pharm. 140: 155-68; Remmele et al. (1997) Pharm. Res. 15: 200-8; Gupta et al. (2003) AAPS PharmSci. 5E8: 2003; Bedu-Addo et al. (2004) Pharm. Res. 21: 1353-61; Zhang et al. (2004) J. Pharm. Sci. 93: 3076-89). Formulation studies suggest that a Fab Tm has implication for long-term physical stability of a corresponding mAb.

[0259] In some exemplary embodiments, strategic introduction of disulfide bonds can stabilize monomeric and multisubunit proteins, play a role in enhancing thermal stability of antibodies.

[0260] In some exemplary embodiments, strategic introduction of π-stacking interactions with aromatic amino acids (AAs) like tryptophan (TRP), tyrosine (TYR), phenylalanine (PHE) and histidine (HIS), play a role in enhancing thermal stability of antibodies.

[0261] In some embodiments, strategic introduction of salt bridges occurring between amino acid side-chains with opposite positive or negative full-electron charges, namely, (at neutral pH) Glu or Asp vs Arg or Lys, enhance the stability of proteins, particularly antibodies.

[0262] In some exemplary embodiments, the monospecific antibody or the biparatopic antibody comprise one or more thermal stability enhancing modifications. In some embodiments, the thermal stability enhancing modification is introduction of a cysteine residue. In some embodiments, the biparatopic antibody comprises cysteine at position 559 of SEQ ID NO: 12 and at position 630 of SEQ ID NO: 12, to enhance the thermal stability.

[0263] In some embodiments, the Tm of exemplary biparatopic antibodies is greater than 65°C. In some embodiments, the Tm of exemplary biparatopic antibodies is greater than 60°C. the Tm of exemplary biparatopic antibodies is greater than 55°C. In some embodiments, the Tm of exemplary biparatopic antibodies is greater than 50°C.

[0264] Some types of biparatopic antibody molecules are produced by crosslinking Site I and Site II binding domains or antigen binding fragments to create bispecific antibodies. Suitable crosslinkers include those that are heterobifunctional, having two distinctly reactive groups separated by an appropriate spacer (*e.g.,* m-maleimidobenzoyl-N-hydroxysuccinimide ester) or homobifunctional (*e.g.,* disuccinimidyl suberate). Such linkers are available from Pierce Chemical Company, Rockford, Ill.

[0265] In some embodiments, peptide linkers are used to link scFv or single chain antibodies to the Fc domain of the Fab. Several Examples of suitable linkers include a single glycine (G) residue; a diglycine peptide (GG); a tripeptide (GGG); a peptide with four glycine residues (GGGG; SEQ ID NO: 26); a peptide with five glycine residues (GGGGG; SEQ ID NO: 27); a peptide with six glycine residues (GGGGGG; SEQ ID NO: 28); a peptide with seven

glycine residues (GGGGGGG; SEQ ID NO: 29); a peptide with eight glycine residues (GGGGGGGG; SEQ ID NO: 30). Other combinations of amino acid residues may be used such as the peptide GGGGS (SEQ ID NO: 31), the peptide GGGGSGGGGS (SEQ ID NO: 32), the peptide GGGGSGGGGSGGGGS (SEQ ID NO: 33), the peptide GGGGSGGGGSGGGGSGGGGS (SEQ ID NO: 34), the peptide GGSGSSGSGG (SEQ ID NO: 35), QRIEG (SEQ ID NO: 36) and the peptide GQPKAAP (SEQ ID NO: 37). Other suitable linkers include a single Ser, and Val residue; the dipeptide RTQP, SS, TK, SL, TKGPS, TVAAP, QPKAA. The examples listed above are not intended to limit the scope of the disclosure in any way, and linkers comprising randomly selected amino acids selected from the group consisting of valine, leucine, isoleucine, serine, threonine, lysine, arginine, histidine, aspartate, glutamate, asparagine, glutamine, glycine, and proline have been shown to be suitable in the binding proteins. For additional descriptions of linker sequences, see, e.g., WO2012135345.

[0266] The identity and sequence of amino acid residues in the linker may vary depending on the type of secondary structural element necessary to achieve in the linker. For example, glycine, serine, and alanine are best for linkers having maximum flexibility. Some combination of glycine, proline, threonine, and serine are useful if a more rigid and extended linker is necessary. Any amino acid residue may be considered as a linker in combination with other amino acid residues to construct larger peptide linkers as necessary depending on the desired properties.

Design of Fc variants

[0267] In some aspects the monospecific and multispecific (including bispecific or biparatopic) C5aR1 antibodies presented herein comprise variations or mutations in the Fc region. In some embodiments, the Fc variants or mutants reduce the ability to undergo Fab arm exchange for the production of stable IgGl or IgG4 bispecific antibodies. (*See* Stubenrauch et al., Drug Metabolism and Disposition 38, 84-91 (2010)). In a particular embodiment the Fc domain is an IgG1 Fc domain. In another embodiment the Fc domain is an IgG4 Fc domain. In a more specific embodiment, the Fc domain is an IgG4 Fc domain comprising an amino acid substitution at position S228 (Kabat numbering), particularly the amino acid substitution S228P.

[0268] In some embodiments, the Fc region comprises a human IgG4 Fc region comprising one or more mutations selected from the group consisting of S228P, L234V, L235A, G237A, D265G, A330S, P331S, L328R, H268A and N297Q mutations (as designated according to Kabat, et al. (1991)). In some instances, a human IgG4 Fc variant has up to 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutation(s) in total as compared to wild-type human IgG4 sequence. In one embodiment, the Fc region comprises F234V, L235E, and D265G mutations. In some embodiments, the Fc region comprises F234V, L235E, D265G, and S228P mutations.

[0269] In some embodiments, the Fc variant exhibits reduced binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits a reduction of phagocytosis compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated phagocytosis compared to the wild-type human IgG Fc region.

[0270] Antibody-dependent cell-mediated cytotoxicity, which is also referred to herein as ADCC, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells and neutrophils) enabling these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell. Antibody-dependent cell-mediated phagocytosis, which is also referred to herein as ADCP, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain phagocytic cells (e.g., macrophages) enabling these phagocytic effector cells to bind specifically to an antigen-bearing target cell and subsequently engulf and digest the target cell. Ligand-specific high-affinity IgG antibodies directed to the surface of target cells can stimulate the cytotoxic or phagocytic cells and can be used for such killing. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit reduced ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in ADCC or ADCP compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, antibodies comprising an Fc variant as described herein exhibit ablated ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region.

[0271] In some embodiments, the Fc variant exhibits reduced binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated binding to an Fc receptor of the subject compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits a reduction of phagocytosis compared to the wild-type human IgG Fc region. In some embodiments, the Fc variant exhibits ablated phagocytosis compared to the wild-type human IgG Fc region.

[0272] Antibody-dependent cell-mediated cytotoxicity, which is also referred to herein as ADCC, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs) present on certain cytotoxic cells (e.g., Natural Killer (NK) cells and neutrophils) enabling these cytotoxic effector cells to bind specifically to an antigen-bearing target cell and subsequently kill the target cell. Antibody-dependent cell-mediated phagocytosis, which is also referred to herein as ADCP, refers to a form of cytotoxicity in which secreted Ig bound onto Fc receptors (FcRs for e.g., FcγRI, FcγRIIa, FcγRIIb, FcγRIIIa and/or FcγRIIIb) present on certain phagocytic cells (e.g., macrophages) enabling these phagocytic effector cells to bind specifically to an antigen-bearing target cell and subsequently engulf and digest the target cell. Ligand-specific high-affinity IgG antibodies directed to the surface of target cells can stimulate the cytotoxic or phagocytic cells and can be used for such killing. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit reduced ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, polypeptide constructs comprising an Fc variant as described herein exhibit at least a 5%, 10%, 15%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90% or greater reduction in ADCC or ADCP compared to a polypeptide construct comprising a wild-type Fc region. In some embodiments, antibodies comprising an Fc variant as described herein exhibit ablated ADCC or ADCP as compared to a polypeptide construct comprising a wild-type Fc region.

[0273] An exemplary monospecific site II (SEQ ID NO: 3) binding antibodies, comprising Fc variants is also referred in this disclosure as c2139-Fcmod. An exemplary biparatopic antibody comprising Fc variants is also referred in this disclosure as c2137-e1711-Fcmod. The sequences of the monospecific and biparatopic antibody comprising the Fc variants are set forth in **Table 6**.

Table 6 – Sequences of Fc modified exemplary monospecific and biparatopic antibodies.

Heavy chain	Light chain
c2139-$F_c$mod	
QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYWMHWVRQAPGQGLEWMGYLNPSSGYTKY AQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCTRSGGDNYGNPYYFDRWGQGTTVT VSSASTKGPSVFPLAPCSRSTSESTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDH KPSNTKVDKRVESKYGPPCPPCPAPEVEGG PSVFLFPPKPKDTLMISRTPEVTCVVVGVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK **(SEQ ID NO: 69)**	DVQMTQSPSSLSASVGDRVTITCRASQSIVH SNGNTYLHWYQQKPGKAPKFLIYKVSNRFSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYC AQYTLVPLTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC **(SEQ ID NO: 70)**
c2137-$F_c$mod	
QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYWMHWVRQAPGQGLEWMGYLNPSSGYTKY AQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCTRSGGDNYGNPYYFDRWGQGTTVT VSSASTKGPSVFPLAPCSRSTSESTAALGC LVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVVTVPSSSLGTKTYTCNVDH KPSNTKVDKRVESKYGPPCPPCPAPEVEGG PSVFLFPPKPKDTLMISRTPEVTCVVVGVS QEDPEVQFNWYVDGVEVHNAKTKPREEQFN STYRVVSVLTVLHQDWLNGKEYKCKVSNKG LPSSIEKTISKAKGQPREPQVYTLPPSQEE MTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSRLTVDKSRW QEGNVFSCSVMHEALHNHYTQKSLSLSLGK **(SEQ ID NO: 69)**	DVQMTQSPSSLSASVGDRVTITCRASQSIVH SNGNTYLHWYQQKPGKAPKFLIYKVSNRFSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYC AQSTLVPLTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC **(SEQ ID NO: 79)**
e1711-$F_c$mod	
EVQLVESGGGLIQPGGSLRLSCAASGFTFN AYAMSWVRQAPGKGLEWVSSISTGGNTYYA DSVKGRFTISRDNSKNTLYLQMNSLRAEDT AVYYCTRGYQRFSGFAYWGQGTLVTVSSAS TKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGL YSLSSVVTVPSSSLGTKTYTCNVDHKPSNT KVDKRVESKYGPPCPPCPAPEVEGGPSVFL FPPKPKDTLMISRTPEVTCVVVGVSQEDPE VQFNWYVDGVEVHNAKTKPREEQFNSTYRV VSVLTVLHQDWLNGKEYKCKVSNKGLPSSI	EIVLTQSPATLSLSPGERATLSCRSSQSLVH SNGNTYLNWYQQKPGQAPRLLIYKVSNRLSG IPARFSGSGSGTDFTLTISSLEPEDFAVYYC SQSTHVPYTFGQGTKLEIKRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKV DNALQSGNSQESVTEQDSKDSTYSLSSTLTL SKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC **(SEQ ID NO: 81)**

EKTISKAKGQPREPQVYTLPPSQEEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYK TTPPVLDSDGSFFLYSRLTVDKSRWQEGNV FSCSVMHEALHNHYTQKSLSLSLGK **(SEQ ID NO: 80)**	
c2137-e1711-Fcmod	
QVQLVQSGAEVKKPGASVKVSCKASGYTFTN YWMHWVRQAPGQGLEWMGYLNPSSGYTKYAQ KFQGRVTMTRDTSTSTVYMELSSLRSEDTAV YYCTRSGGDNYGNPYYFDRWGQGTTVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEVEGGPSVFLFPP KPKDTLMISRTPEVTCVVVGVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGGGGGSEIVLTQSPATL SLSPGERATLSCRSSQSLVHSNGNTYLNWYQ QKPGQAPRLLIYKVSNRLSGIPARFSGSGSG TDFTLTISSLEPEDFAVYYCSQSTHVPYTFG CGTKLEIKGGGGSGGGGSGGGGSGGGGSEVQ LVESGGGLIQPGGSLRLSCAASGFTFNAYAM SWVRQAPGKCLEWVSSISTGGNTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCT RGYQRFSGFAYWGQGTLVTVSS **(SEQ ID NO: 71)**	DVQMTQSPSSLSASVGDRVTITCRASQSIV HSNGNTYLHWYQQKPGKAPKFLIYKVSNRF SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCAQSTLVPLTFGQGTKLEIKRTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC **(SEQ ID NO: 72)**
c2139-e1711-Fcmod	
QVQLVQSGAEVKKPGASVKVSCKASGYTFTN YWMHWVRQAPGQGLEWMGYLNPSSGYTKYAQ KFQGRVTMTRDTSTSTVYMELSSLRSEDTAV YYCTRSGGDNYGNPYYFDRWGQGTTVTVSSA STKGPSVFPLAPCSRSTSESTAALGCLVKDY FPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVVTVPSSSLGTKTYTCNVDHKPSNTKV DKRVESKYGPPCPPCPAPEVEGGPSVFLFPP KPKDTLMISRTPEVTCVVVGVSQEDPEVQFN WYVDGVEVHNAKTKPREEQFNSTYRVVSVLT VLHQDWLNGKEYKCKVSNKGLPSSIEKTISK AKGQPREPQVYTLPPSQEEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSRLTVDKSRWQEGNVFSCSVMHEA LHNHYTQKSLSLSLGGGGGSEIVLTQSPATL SLSPGERATLSCRSSQSLVHSNGNTYLNWYQ	DVQMTQSPSSLSASVGDRVTITCRASQSIV HSNGNTYLHWYQQKPGKAPKFLIYKVSNRF SGVPSRFSGSGSGTDFTLTISSLQPEDFAT YYCAQYTLVPLTFGQGTKLEIKRTVAAPSV FIFPPSDEQLKSGTASVVCLLNNFYPREAK VQWKVDNALQSGNSQESVTEQDSKDSTYSL SSTLTLSKADYEKHKVYACEVTHQGLSSPV TKSFNRGEC **(SEQ ID NO: 82)**

QKPGQAPRLLIYKVSNRLSGIPARFSGSGSG TDFTLTISSLEPEDFAVYYCSQSTHVPYTFG CGTKLEIKGGGGSGGGGSGGGGSGGGGSEVQ LVESGGGLIQPGGSLRLSCAASGFTFNAYAM SWVRQAPGKCLEWVSSISTGGNTYYADSVKG RFTISRDNSKNTLYLQMNSLRAEDTAVYYCT RGYQRFSGFAYWGQGTLVTVSS **(SEQ ID NO: 71)**	
Variable region	
VL of c2139-Fcmod	***VH of c2139-Fcmod***
DVQMTQSPSSLSASVGDRVTITCRASQSIVH SNGNTYLHWYQQKPGKAPKFLIYKVSNRFSG VPSRFSGSGSGTDFTLTISSLQPEDFATYYC AQYTLVPLTFGQGTKLEIK **(SEQ ID NO: 73)**	QVQLVQSGAEVKKPGASVKVSCKASGYTFT NYWMHWVRQAPGQGLEWMGYLNPSSGYTKY AQKFQGRVTMTRDTSTSTVYMELSSLRSED TAVYYCTRSGGDNYGNPYYFDRWGQGTTVT VSS **(SEQ ID NO: 74)**

[0274] In some embodiments, the monospecific anti-C5aR1 antibody comprises a heavy chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 69.

[0275] In some embodiments, the monospecific anti-C5aR1 antibody comprises a light chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 70.

[0276] In some embodiments, the biparatopic anti-C5aR1 antibody comprises a heavy chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 71.

[0277] In some embodiments, the biparatopic anti-C5aR1 antibody comprises a heavy chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 72.

[0278] In some embodiments, the monospecific anti-C5aR1 antibody comprises a light chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 79.

[0279] In some embodiments, the monospecific anti-C5aR1 antibody comprises a light chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 81.

[0280] In some embodiments, the monospecific anti-C5aR1 antibody comprises a light chain amino acid sequence having at least 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to SEQ ID NO: 82.

Use of monospecific and biparatopic C5aR1 antagonists for treatment of diseases

[0281] Described herein are methods of treating a disease associated with C5a/C5aR1 axis dysfunctions. Accordingly, in some embodiments, the monospecific and/or biparatopic antibodies against C5aR1 described herein are suitable for treating a subject that has a dysfunction associated with C5a/C5aR1 axis, such as ANCA-associated vasculitis.

[0282] Exemplary disorders or conditions that can be treated or prevented by the antibody molecules described herein include, but are not limited to, a C5aR1-associated disorder or a C5-associated disorder. In an embodiment, the disorder is associated with neutrophil recruitment, activation, and/or NETosis. In an embodiment, the disorder is associated with complement system activation and/or coagulation system activation. In an embodiment, the disorder is associated with a C5aR-mediated inflammatory response. In an embodiment, the disorder is associated with monocyte chemoattractant protein-1 (MCP-1) and/or renal inflammation. In an embodiment, the disorder is associated with chemotaxis (*e.g.*, chemotaxis priming). In an embodiment, the disorder is associated with endothelium injury.

[0283] The method of treating includes administering to the subject in need thereof antibodies described herein. In an embodiment, the exemplary disorders, for example, a C5aR1-associated disorder, can be treated using an antibody capable of binding one or more amino acid residues of C5aR1 at Site II (SEQ ID NO: 3).

[0284] In an embodiment, the exemplary disorders, for example, a C5aR1-associated disorder, can be treated using an antibody capable of competing with an antibody binding to C5aR1 at Site I (SEQ ID NO: 1 or SEQ ID NO: 2) or at Site II (SEQ ID NO: 3).

[0285] In an embodiment, the exemplary disorders, for example, a C5aR1-associated disorder, can be treated using an antibody capable of competing with an antibody binding to C5aR1 at Site I (SEQ ID NO: 1 or SEQ ID NO: 2) and at Site II (SEQ ID NO: 3).

[0286] The monospecific and/or biparatopic antibodies described herein can be used to treat any disease associated with dysfunction associated with C5a/C5aR1 axis.

[0287] In some embodiments, the exemplary disorders, for example, a C5aR1-associated disorder, can be treated using a monospecific or biparatopic antibody capable of binding to C5aR1, wherein the antibody binds C5aR1 with an affinity of 10pM to 50nM.

[0288] In some embodiments, the antibodies can be administered to a subject in need thereof, intravenously, subcutaneously, intradermally or intramuscularly.

[0289] In some embodiments, a nucleic acid encoding the monospecific or biparatopic antibodies described herein can be administered to a subject in need thereof using an appropriate delivery method. Several methods for delivering nucleic acids are known in literature. For example, a rAAV vector encoding the monospecific or biparatopic antibodies described herein can be administered to a subject administered by intravenous, intraperitoneal, subcutaneous, or intradermal administration. In some embodiments, the delivery of the nucleic acid encoding the antibody can be achieved using a "gene gun", a biolistic particle delivery system or a non-viral lipid nanoparticle.

[0290] In some embodiments, the monospecific or biparatopic antibodies described herein can be administered to a subject in need thereof in combination with an additional therapeutic. In some embodiments, the additional therapeutic is a small molecule, such as avacopan, corticosteroids or immunosuppressive drugs. Exemplary corticosteroids include but are not limited to, prednisolone, hydrocortisone, prednisone, dexamethasone or cortisone. Exemplary immunosuppressive drugs include, but are not limited to methotrexate, Azathioprine, Mycophenolate mofetil or cyclophosphamide.

[0291] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan monotherapy in alleviating neutropenia, *in vivo*.

[0292] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan in antagonizing C5aR1 in the presence of 100nM of greater concentrations of C5a.

[0293] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan in inhibiting Gα signaling, *in vitro*.

[0294] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan in inhibiting calcium signaling, *in vitro* and *in vivo*.

[0295] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan in inhibiting neutrophil chemotaxis, *in vitro* and *in vivo*.

[0296] In some embodiments, the monospecific or biparatopic antibodies described herein are stable in serum for up to 500 hours post injection.

[0297] In some embodiments, the monospecific or biparatopic antibodies described herein are better than avacopan in inhibiting CD11b expression, *in vitro* and *in vivo*.

Nucleic Acids, Vectors and Methods of Manufacturing

[0298] The present disclosure also features nucleic acids comprising nucleotide sequences that encode the antibody molecules (e.g., heavy and light chain variable regions and CDRs of the antibody molecules), as described herein.

[0299] For example, the present disclosure features a first and second nucleic acid encoding heavy and light chain variable regions, respectively, of an antibody molecule chosen from one or more of the antibody molecules disclosed herein, e.g., an antibody molecule of Table 1C, or a portion of an antibody molecule, e.g., the variable regions of any of the antibodies disclosed herein. The nucleic acid can comprise a nucleotide sequence encoding any one of the amino acid sequences in the tables herein, or a sequence substantially identical thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, or which differs by no more than 3, 6, 15, 30, or 45 nucleotides from the sequences shown in the tables herein).

[0300] In certain embodiments, the nucleic acid comprises a nucleotide sequence encoding at least one, two, or three CDRs from a heavy chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions). In an embodiment, the nucleic acid comprises a nucleotide sequence encoding at least one, two, or three CDRs from a light chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions). In an embodiment, the nucleic acid comprises a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs from heavy and light chain variable regions having an amino acid

sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions).

[0301] The nucleic acids disclosed herein include deoxyribonucleotides or ribonucleotides, or analogs thereof. The polynucleotide may be either single-stranded or double-stranded, and if single-stranded may be the coding strand or non-coding (antisense) strand. A polynucleotide may comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. The sequence of nucleotides may be interrupted by non-nucleotide components. A polynucleotide may be further modified after polymerization, such as by conjugation with a labeling component. The nucleic acid may be a recombinant polynucleotide, or a polynucleotide of genomic, cDNA, semisynthetic, or synthetic origin which either does not occur in nature or is linked to another polynucleotide in a non-natural arrangement.

[0302] In some aspects, the application features host cells and vectors containing the nucleic acids described herein. The nucleic acids may be present in a single vector or separate vectors present in the same host cell or separate host cell, as described in more detail below.

Vectors

[0303] Further provided herein are vectors that comprise nucleotide sequences encoding the antibody molecules (e.g., heavy and light chain variable regions and CDRs of the antibody molecules), as described herein.

[0304] In an embodiment, the vector comprises a nucleic acid described herein. For example, the vector can comprises a first and second nucleic acid encoding heavy and light chain variable regions, respectively, of an antibody molecule chosen from one or more of the antibody molecules disclosed herein, e.g., an antibody molecule described herein, or a portion of an antibody molecule, e.g., the variable regions of any of Tables 1-4.

[0305] In certain embodiments, the vector comprises a nucleotide sequence encoding at least one, two, or three CDRs from a heavy chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions). In an embodiment, the vector comprises a

nucleotide sequence encoding at least one, two, or three CDRs from a light chain variable region having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions). In an embodiment, the vector comprises a nucleotide sequence encoding at least one, two, three, four, five, or six CDRs from heavy and light chain variable regions having an amino acid sequence as set forth in the tables herein, or a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or having one or more substitutions, e.g., conserved substitutions).

[0306] The vectors include, but are not limited to, a virus, plasmid, cosmid, lambda phage or a yeast artificial chromosome (YAC). Numerous vector systems can be employed. For example, one class of vectors utilizes DNA elements which are derived from animal viruses such as, for example, bovine papilloma virus, polyoma virus, adenovirus, vaccinia virus, baculovirus, retroviruses (Rous Sarcoma Virus, MMTV or MOMLV) or SV40 virus. Another class of vectors utilizes RNA elements derived from RNA viruses such as Semliki Forest virus, Eastern Equine Encephalitis virus and Flaviviruses.

[0307] Additionally, cells which have stably integrated the DNA into their chromosomes may be selected by introducing one or more markers which allow for the selection of transfected host cells. The marker may provide, for example, prototropy to an auxotrophic host, biocide resistance (e.g., antibiotics), or resistance to heavy metals such as copper, or the like. The selectable marker gene can be either directly linked to the DNA sequences to be expressed or introduced into the same cell by cotransformation. Additional elements may also be needed for optimal synthesis of mRNA. These elements may include splice signals, as well as transcriptional promoters, enhancers, and termination signals.

[0308] Once the expression vector or DNA sequence containing the constructs has been prepared for expression, the expression vectors may be transfected or introduced into an appropriate host cell. Various techniques may be employed to achieve this, such as, for example, protoplast fusion, calcium phosphate precipitation, electroporation, retroviral transduction, viral transfection, gene gun, lipid-based transfection or other conventional techniques. In the case of protoplast fusion, the cells are grown in media and screened for the appropriate activity.

[0309] Methods and conditions for culturing the resulting transfected cells and for recovering the antibody molecule produced are known to those skilled in the art and may be varied or optimized depending upon the specific expression vector and mammalian host cell employed, based upon the present description.

Cells

[0310] The present disclosure also provides cells (e.g., host cells) comprising a nucleic acid encoding an antibody molecule as described herein. For example, the host cells may comprise a nucleic acid molecule having a nucleotide sequence encoding an amino acid sequence described in any of Tables 1-5, a sequence substantially homologous thereto (e.g., a sequence at least about 85%, 90%, 95%, 99% or more identical thereto, and/or capable of hybridizing under the stringency conditions described herein), or a portion of one of said nucleic acids.

[0311] In an embodiment, the host cells are genetically engineered to comprise nucleic acids encoding the antibody molecule described herein.

[0312] In certain embodiments, the host cells are genetically engineered by using an expression cassette. The phrase "expression cassette," refers to nucleotide sequences, which are capable of affecting expression of a gene in hosts compatible with such sequences. Such cassettes may include a promoter, an open reading frame with or without introns, and a termination signal. Additional factors necessary or helpful in effecting expression may also be used, such as, for example, an inducible promoter.

[0313] The disclosure also provides host cells comprising the vectors described herein.

[0314] The cell can be, but is not limited to, a eukaryotic cell, a bacterial cell, an insect cell, or a human cell. Suitable eukaryotic cells include, but are not limited to, Vero cells, HeLa cells, COS cells, CHO cells, HEK293 cells, BHK cells and MDCKII cells. Suitable insect cells include, but are not limited to, *Sf*9 cells. In an embodiment, the cell (e.g., host cell) is an isolated cell.

Methods of Administration

[0315] Compositions of the invention can be formulated in any suitable form, such as liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible

solutions), dispersions or suspensions, and the like. The optimal form for any composition depends on the intended mode of administration, the nature of the composition or combination, and therapeutic application or other intended use. A typical mode for delivery for a composition of the invention is by parenteral administration (e.g., intravenous administration). In one aspect, a composition of the invention is administered to a human patient by intravenous infusion or injection.

[0316] In some aspects, this disclosure provides compositions, e.g., pharmaceutically acceptable compositions, which include an antibody molecule described herein, formulated together with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" includes any and all solvents, dispersion media, isotonic and absorption delaying agents, and the like that are physiologically compatible. The carrier can be suitable for intravenous, intramuscular, subcutaneous, parenteral, rectal, spinal or epidermal administration (e.g., by injection or infusion).

[0317] The phrases "parenteral administration" and "administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Therapeutic compositions typically should be sterile and stable under the conditions of manufacture and storage. The composition can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable to high antibody concentration. Sterile injectable solutions can be prepared by incorporating the active compound (i.e., antibody or antibody portion) in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients 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 that yields a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of

dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition an agent that delays absorption, for example, monostearate salts and gelatin. The antibody molecules described herein can be administered by a variety of methods. Several are known in the art, and for many therapeutic, prophylactic, or diagnostic applications, an appropriate route/mode of administration is intravenous injection or infusion. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results.

EXAMPLES

[0318] Other features, objects, and advantages of the present disclosure are apparent in the examples that follow. It should be understood, however, that the examples, while indicating embodiments of the present disclosure, are given by way of illustration only, not limitation. Various changes and modifications within the scope of the disclosure will become apparent to those skilled in the art from the examples.

Example 1. Kinetic analyses of C5aR1 antagonistic humanized antibodies

[0319] This Example describes the binding affinity and specificity of Site II monospecific antibody and biparatopic antibodies described herein, to C5aR1 and C5aR2.

(a) Binding affinity

[0320] The binding affinity of the antibodies to the target receptor, C5aR1, was determined using an ELISA assay. C5aR1 VLP were immobilized to a MaxiSorp ELISA plate at a concentration of 30 µg/mL and incubated overnight at 4°C. The following morning, plates were washed 3 times with 1x PBS and plates were blocked for 30 minutes with 100 µL of PBSA (1x PBS with 3% BSA). A serial titration of anti-C5aR1 antibody was performed in the presence of PBSA and incubated for 1 hour at room temperature. Plates were washed 6 times with PBSA. Anti-human-HRP was diluted in PBSA, added to all wells, and incubated for 45 minutes at room temperature. Plates were washed 6 times with PBS. TMB substrate was added to all wells and incubated for 10 minutes before the addition of stop solution (0.1 M sulfuric acid). Absorbance at 450 nm was measured on a standard plate reader. A four-parameter curve fit was used to

generate the EC50 value of the antibody titration in nM. Affinity curves of a monospecific c2139 and a biparatopic c2137-e1711 antibodies are illustrated in **FIG. 4A**.

[0321] It was observed that under the conditions described above, the monospecific C5aR1 antibody, bound C5aR1 with an affinity of about 0.16nM and the biparatopic antibody bound C5aR1 with an affinity of about 0.22nM.

[0322] In another setting, the binding of site II antibody, c2139 and biparatopic antibody c2137-e1711 from two different antibody batches were assayed for binding to U937-C5aR1 cells (**FIG. 4B**) and human neutrophils (**FIG. 4C**). The EC50 of U937-C5aR1 binding to different protein batches of anti-C5aR1 antibody (c2139 and c2137-e1711) different shown in **Table 6**.

Table 6. EC50 of U937-C5aR1 binding to exemplary C5aR1 antibody

Antibody	EC50 (nM)	R Squared
c2139 Prep 1	3.64	0.99
c2139 Prep 2	3.60	0.99
c2137-e1711 Prep 1	2.47	0.98
c2137-e1711 Prep 2	2.25	0.98
motavizumab	No Binding	n/a

[0323] The EC50 of human neutrophil binding to different protein batches of anti-C5aR1 antibody (c2139 and c2137-e1711) different shown in **Table 7**.

Table 7. EC50 of human neutrophil binding to exemplary C5aR1 antibody

Antibody	EC50 (nM)	R Squared
c2139 Prep 1	1.64	0.99
C2139 Prep 2	1.66	0.99
c2137-e1711 Prep 1	1.07	0.99
c2137-e1711 Prep 2	1.05	0.99
motavizumab	No Binding	n/a

[0324] A non-C5aR1 antibody, motavizumab was used as a control. It was observed that both Site II antibody, c2139 and biparatopic antibody c2137-e1711 bound U937 and human neutrophils across multiple antibody preparation lots.

(b) Binding specificity

[0325] The specificity of the C5aR1 antagonistic antibodies was determined by measuring the affinity of anti-C5aR1 antibodies to C5aR2. C5aR2 VLP were immobilized to a MaxiSorp ELISA plate at a concentration of 30 μg/mL and incubated overnight at 4°C. The following morning, plates were washed 3 times with 1x PBS and plates were blocked for 30 minutes with 100 μL of PBSA (1x PBS with 3% BSA). A serial titration of anti-C5aR1 antibody was performed in the presence of PBSA and incubated for 1 hour at room temperature. Plates were washed 6 times with PBSA. Anti-human-HRP was diluted in PBSA, added to all wells, and incubated for 45 minutes at room temperature. Plates were washed 6 times with PBS. TMB substrate was added to all wells and incubated for 10 minutes before the addition of stop solution (0.1 M sulfuric acid). Absorbance at 450 nm was measured on a standard plate reader. A four-parameter curve fit was used to generate the EC50 value of the antibody titration in nM.

[0326] A binding data of a monospecific and a biparatopic antibody is illustrated in **FIG. 5**. It was observed that under the conditions described above the exemplary C5aR1 antagonistic antibodies did not bind C5aR2. Overall, the data in this example showed that anti-C5aR1 antibodies of the present invention bind C5aR1 with high affinity, and with unmeasurable affinity to C5aR2, illustrating that the C5aR1 antibodies are indeed specific for C5aR1.

Example 2. Inhibition of Gα signaling

[0327] This Example describes the functional aspect of C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of Gα signaling using a GeneBLazer assay.

[0328] The GeneBLAzer assay kit and C5aR1 cell line were commercially available from Thermo Fisher (Catalog # K1544). The assay was performed as recommend by the manufacturer. Briefly, antibodies or antagonists were incubated for 30 minutes at increasing concentrations at 37°C. C5a was then added to the cells and incubated at 37°C for an additional 4-5 hours. Beta-lactamase substrate was then added and incubated for 2 hours at room temperature. Fluorescent measurements for each well with an excitation/emission of 409/460 (blue) and 409/530 (green) were measured. An increase in the blue to green ratio was proportional to C5aR1 activation and was used to calculate the percent of activation for the cells

in each well. The C5aR1 activation profile was monitored under increasing antagonist concentration. The IC50 was determined by plotting the percentage of signal against concentration of the antagonist.

[0329] A Gα signaling assay of a monospecific and a biparatopic antibody is illustrated in **FIG. 6A** as a function of antibody concentration with 10nM C5a. In another setting, a known C5aR antibody, an anti-C5aR1 control Ab is used as a positive control for inhibition of Gα signaling (**FIG. 6C**).

[0330] A Gα signaling assay of a monospecific and a biparatopic antibody is illustrated in **FIG. 6B** as a function of antibody concentration with 100nM C5a. In another setting, a known C5aR antibody, an anti-C5aR1 control Ab is used as a positive control for inhibition of Gα signaling (**FIG. 6D**). It was observed that the IC50 of monospecific C5aR1 antagonist was 0.14 nM with 10nM C5a and 0.19 nM with 100nM C5a. The IC50 of bispecific C5aR1 antagonist was 0.27 nM with 10 nM C5a and 0.28 nM with 100nM C5a. It was observed that the exemplary antibodies retained superior inhibition compared to Avacopan and an anti-C5aR1 control Ab at all concentrations of C5a tested.

Example 3. Inhibition of Calcium signaling

[0331] This Example describes the functional aspect of C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of C5a mediated calcium signaling, using calcium flux assays.

[0332] The potency of the antibodies was assessed for their ability to inhibit intracellular calcium release in C5aR1 expressing stable C5aR1-U937 cells or neutrophils isolated from whole blood in the presence of C5a. Calcium flux assays using calcium sensitive dye were utilized to detect cytosolic changes in calcium concentration. Cells were incubated with esterified (inactive) calcium dye. The dye penetrated the cell membrane and became active once inside the cell. The active form of the dye became fluorescent after binding to intracellular calcium and the fluorescence was used to determine C5aR1 signaling in response to C5a addition. Specifically, the stable C5aR1-U937 or neutrophils isolated from whole blood were stained with Fluo-4 Direct calcium Assay kit from Thermo Fisher for 1 hour at 37°C. Next, antibody or antagonist was incubated with the cells for 30 minutes. The basal fluorescence with

an excitation at 494 nm and emission at 516 nm was measured for 15 seconds. C5a was added to the cells and the fluorescence was measured over a span of four minutes. The basal read from before C5a stimulation and the max signal after C5a stimulation were used to calculate the response ratio. The calcium flux assays were performed using engineered cells stably expressing C5aR1 as well as using human neutrophils.

[0333] An inhibition of C5a-meditated calcium signaling assay of a monospecific (**FIG. 7A**) and a biparatopic antagonistic antibody (**FIG. 7B**) is illustrated in as a plotted as calcium flux as a function C5a concentration and with increasing antibody concentration. Further, inhibition of C5a-meditated calcium signaling with a known C5aR1 antagonist, Avacopan, is illustrated using the protocol as described above in **FIG. 7C**. It was observed that the tested antibodies were better at inhibition of calcium signaling than avacopan.

Example 4. Inhibition of Neutrophil chemotaxis

[0334] This Example describes the functional aspect of C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of neutrophil chemotaxis, which is known to be induced by C5aR1 activity, using a Boyden Chamber.

[0335] First, C5aR1-U937 stable cells were seeded into the top chamber of a 96 Transwell plate in the presence of no antibody (antagonist), or of 1, 10, or 100 nM each antibody (antagonist). The bottom chamber contained a half-log titration of C5a (chemoattractant) ranging from 10^{-6} to $10^{-9.5}$ M. The top and the bottom chambers were separated by a membrane. C5a is expected to induce migration of U937 cells. However, use of a C5aR1 antagonist is expected to inhibit this migration. Increasing concentration of the exemplary antibodies were used to determine a dose-dependent inhibition of chemotaxis. Further, increasing concentration of the chemoattractant (C5a) was used to determine if the inhibition of chemotaxis was surmountable by using high concentration of C5a. In a parallel setting, a known C5aR1 inhibitor, Avacopan was also used to evaluate the inhibition of chemotaxis of neutrophils and surmountability of the chemotaxis in the presence of excess C5a.

[0336] Second, human neutrophils were seeded into the top chamber of a 96 Transwell plate in the presence of no antibody (antagonist), or of 1, 10, or 100 nM each antibody (antagonist). The bottom chamber contained a half-log titration of C5a (chemoattractant) ranging

from 10^{-6} to $10^{-9.5}$ M. The top and the bottom chambers were separated by a membrane. C5a is expected to induce migration of U937 cells. However, use of a C5aR1 antagonist is expected to inhibit this migration. Increasing concentration of the antibodies were used to determine a dose-dependent inhibition of chemotaxis. Further, increasing concentration of the chemoattractant (C5a) was used to determine if the inhibition of chemotaxis was surmountable by using high concentration of C5a. In a parallel setting, a known C5aR1 inhibitor, Avacopan was also used to evaluate the inhibition of chemotaxis of neutrophils and surmountability of the chemotaxis in the presence of excess C5a.

[0337] **FIG. 8A-8C** shows the amount of fluorescence intensity versus the C5a concentration, in the presence of different concentration of the anti-C5aR1 antibodies, indicating inhibition of chemotaxis by the anti-C5aR1 antibodies. It was observed that the anti-C5aR1 antibodies generally inhibited cell chemotaxis in a dose-dependent manner. Further, it was observed that unlike Avacopan, the inhibition of neutrophil chemotaxis was insurmountable in the presence of excess C5a, by the anti-C5aR1 antibodies.

Example 5. Inhibition of CD11b expression

[0338] This Example describes the functional aspect of C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of CD11b expression.

[0339] CD11b, an integrin receptor, is mobilized to the surface of neutrophils in response to C5a activation and mediates intravascular crawling prior to neutrophil transmigration. CD11 is involved in numerous adhesion-related associations between cells such as monocytes, macrophages, natural killer (NK) cells, and granulocytes.

[0340] **FIG. 9A-9B** shows the percentage of CD11b expression versus the antibody (C5aR1 antagonist) concentration, in the presence of 100nM C5a. It was observed that the antibodies potently inhibited CD11b across the entire range of expected physiological concentration of C5a. **Table 8** shows the IC50 of c2139 and c2137-e1711 in the presence of 100nM C5a.

Table 8. IC50 of CD11b expression inhibition using exemplary C5aR1 antibodies

Antagonist	**IC50 (nM)**
	100 nM C5a

C2139	**1.85**
C2137-e1711	**1.47**

[0341] In another setting, 10nM and 100nM an anti-C5aR1 control Ab was used as a control to compare the inhibition of the anti-C5aR1 antibodies disclosed herein (**FIG. 9C**) in human neutrophils. It was observed further observed that avacopan was only partially potent in inhibition of CD11b. It was further observed that instant antibodies were better inhibitors than an anti-C5aR1 control Ab.

Example 6. Inhibition of β-arrestin recruitment

[0342] This Example describes the functional aspect of C5aR1 monospecific antibody, and biparatopic antibodies described herein, for inhibition of β-arrestin recruitment.

[0343] To characterize the lead mAbs as full antagonists of C5aR1, we aimed to evaluate the potential of the lead mAbs to inhibit β-arrestin2 recruitment. The β-arrestin assay utilized a proprietary two-subunit luciferase reporter system: one subunit is fused to C5aR1 and one subunit is fused to β-arrestin2. When in close proximity, the two subunits formed an enzyme that generates a luminescent signal, used as a proxy for C5aR1-mediated recruitment of β-arrestin2. Exemplary antibodies significantly reduced C5aR1-mediated recruitment of β-arrestin2 at two different C5a concentrations, 1 nM and 10 nM. **FIGs. 10A-10C** are graphs showing the inhibition of the fluorescent signal, after addition of 1nM (**FIG. 10A**), 10nM (**FIG. 10B**) and 100nM (**FIG. 10C**) C5a. **Table 9** shows the IC50 of c2139, c2137-e1711 and avacopan mediated inhibition of β-arrestin.

Table 9. IC50 of monospecific or biparatopic antibody for β-arrestin signaling

Name	10 nM C5a		100 nM C5a	
	IC50	R value	IC50	R value
c2139	1.783 nM	0.995	0.6516 nM	0.999
c2137-e1711	0.766 nM	0.829	0.3598 nM	0.972
Avacopan	9.953 nM	0.818	3.246 nM	0.846

[0344] When compared to the small molecule C5aR1 inhibitor avacopan, exemplary antibodies, c2139 and c2137-e1711 showed superior inhibition of signal, even when avacopan was used at 10-fold higher concentrations than the antibodies. Notably, avacopan lost potency as C5a concentrations increase to 10 nM, whereas c2139 and c2137-e1711 maintain >75% inhibition of β-arrestin2 recruitment. The data suggested that the exemplary C5aR1 antibodies, c2139 and c2137-e1711 inhibit β-arrestin at 10-fold lower concentrations than Avacopan.

Example 7. Inhibition of ROS production by humanized anti-C5aR1 antibodies

[0345] This example shows that exemplary humanized monospecific antibodies (c2139) and exemplary biparatopic antibodies (c2137-e1711) reduced reactive oxygen species (ROS).

[0346] ROS production by neutrophils was detected with a Cellular ROS Detection Assay Kit (Abcam) following manufacturer's manual. The RBC-lysed WB cells were diluted to the concentration of 3 x 10^5 cells in 100 mL buffer. Pre-treatment with ROS inhibitor (N-acetyl-L-cysteine) was carried out for the negative control group at 37°C, 5 % CO2 for 30 minutes. ROS detection antibody then added into the antibody cocktail for flow cytometry-based detection of neutrophil ROS production. ROS inducer (pyocyanin) was added to all groups and incubated for 30 minutes prior to acquisition on the cytometer. **FIG. 11** shows the ROS production in neutrophils in response in ANCA- and ANCA+ groups in response to treatment with anti-C5aR1 antibodies.

[0347] It was observed that c2139 and c2137-e1711 inhibited ANCA induced ROS production.

Example 8. Internalization of humanized anti-C5aR1 antibodies in U937 cells

[0348] This example shows the internalization of humanized monospecific and biparatopic C5aR1 antibodies in hC5ar1-U937 cells.

The humanized monospecific anti-C5aR1 antibody, e1711 and biparatopic antibody, c2139-e1711 were conjugated to pH sensitive dye, which Fluoresces brightly at low pH but is non-fluorescent at neutral pH. The conjugated antibodies were incubated with U937 cells and hC5aR1 knock in U937 cells. **FIG. 12A-12D** show the fluorescence intensity after 24 hours of incubation with each conjugated antibody. **FIG. 12A-FIG. 12B** shows fluorescence intensity of

U037 cells or U937-C5aR1 cells with 10 nM antibodies; and **FIG. 12C – FIG.12D** shows fluorescence intensity of U037 cells or U937-C5aR1 cells with 100 nM antibodies.

Example 9. Cross-reactivity of humanized anti-C5aR1 antibodies

[0349] This example shows the binding of humanized anti-C5aR1 antibody, e1711 and biparatopic antibody, c2139-e1711 to C5aR1 in squirrel monkey and dog.

[0350] Cell surface binding and flow cytometry were performed on half-log titrations of anti-C5aR1 antibodies ranging from at $10^{-7.5}$ to 10^{-12} M. As shown in **FIG. 13A-13B**, the anti-C5aR1 humanized antibodies bound Squirrel monkey and dog C5aR1.

Example 10. Inhibition of Neutropenia in Squirrel Monkeys

[0351] This example shows the inhibition of neutropenia by C5aR1 antibodies in squirrel monkeys. Neutropenia is caused by rapid expression of cell surface CD11b which allows neutrophils to transiently adhere to blood vessel endothelium, thus reducing peripheral neutrophil counts. Squirrel monkey is a physiologically relevant model compared to mouse for evaluation of neutropenia, as neutrophil counts in squirrel monkeys are similar to humans (50 - 70%), mice have only 10-20%. Further, Squirrel monkey allows blood collection at multiple time points possible enabling longer study duration. It was further observed that cross-reacts with squirrel monkey C5aR1, as shown in **FIG. 13A**.

[0352] **FIG. 14A** shows the experiment design in squirrel monkeys to evaluate alleviation of neutropenia. Briefly, three groups of squirrel monkeys were evaluated for inhibition of neutropenia. In the first group, 8 squirrel monkeys were injected with PBS (vehicle), intravenously. In the second group 8 squirrel monkeys were injected with exemplary antibodies at a dose of 10mg/kg, intravenously. In the third group, group 8 squirrel monkeys were injected with avacopan at a dose of 30mg/kg, intravenously. One hour after injection of the PBS, exemplary antibodies or avacopan, 0.1mg/kg of human C5a was injected into the squirrel monkeys. The blood was collected after 1 min, 5 min 15 min, 2 hr and 6hr after injection of human C5a. The percent change in neutrophils and average change in neutrophils were calculated in all three groups and are shown in **FIG. 14B**, as scatter plot. The average change in neutrophils are shown in **FIG. 14C**, as bar graph.

[0353] It was observed that administration of human C5a resulted in rapid transient neutropenia, caused by neutrophil adhesion to endothelial cells. The vehicle (PBS) group demonstrated robust neutropenia with 89% average reduction in neutrophil counts from baseline at 1 min post-C5a injection. Significant inhibition of neutropenia was observed on pre-treatment with exemplary anti-C5aR1 antibodies (23% vs 89% for vehicle). It was further observed that the neutrophil counts starts to resolve between 2 to 6 hours. Neutrophilia observed for Avacopan. No neutrophilia was observed for c2139. Avacopan group also responded with 59% average decrease in neutrophils. It was observed that exemplary anti-C5aR1 antibodies had superior response rate when compared to Avacopan.

Example 11. Inhibition of Neutropenia in human C5aR1 transgenic mice

[0354] This example shows the inhibition of neutropenia by C5aR1 antibodies in transgenic human C5aR1 mice. The exemplary C5aR1 antibodies do not cross-react with mouse C5aR1. Transgenic human C5aR1 (hC5aR1) knock-in mice were generated using CRISPR technology at The Jackson Laboratory.

[0355] **FIG. 15A** shows the experiment design in mice to evaluate alleviation of neutropenia. Briefly, five groups of hC5aR1 mice were evaluated for inhibition of neutropenia. In the first group, 5 hC5aR1 mice were injected with PBS (vehicle), intravenously. In the second group 5 hC5aR1 mice were injected with exemplary monospecific anti-C5aR1 antibody, c2139, at a dose of 20mg/kg, intravenously. In the third group, group 5 hC5aR1 mice were injected with exemplary biparatopic anti-C5aR1 antibody, c2137-e1711, intravenously at a dose of 20mg/kg. In the fourth group, 5 hC5aR1 mice were injected with non-C5aR1 antibody (Motavizumab) intravenously at a dose of 20mg/kg. In the fifth group, 5 hC5aR1 mice were injected with avacopan at a dose of 30mg/kg, intravenously. One hour after injection of the PBS, exemplary antibodies, motavizumab or avacopan, 0.1mg/kg of human C5a was injected into the hC5aR1 mice. The blood was collected after 1 min, 5 min and 2 hr after injection of human C5a. The percent chance in neutrophils and average change in neutrophils were calculated in all three groups and are shown in **FIG. 15B-15C**. The average change in neutrophils are shown in **FIG. 15C**. Neutrophil cell counts were assessed by gating CD45+/CD11b+/Ly6G+ cells.

[0356] It was observed that administration of human C5a resulted in rapid transient neutropenia, caused by neutrophil adhesion to endothelial cells. The vehicle (PBS) group

demonstrated robust neutropenia with 89% average reduction in neutrophil counts from baseline at 1 min post-C5a injection. Significant inhibition of neutropenia was observed on pre-treatment with exemplary anti-C5aR1 antibodies (23% vs 89% for vehicle). Avacopan group also responded with 59% average decrease in neutrophils.

[0357] It was observed that exemplary anti-C5aR1 antibodies had superior response rate when compared to Avacopan. It was observed that administration of human C5a resulted in rapid transient neutropenia in human C5aR1 mice. The vehicle (PBS) group demonstrated robust neutropenia with 76% average reduction in neutrophil counts from baseline at 1 min post-C5a injection. Significant inhibition of neutropenia was observed on pre-treatment with exemplary monospecific C5aR1 antibody (11% vs 89% for vehicle). Complete inhibition was seen for exemplary biparatopic C5aR1 antibody. Avacopan group also responded with 10% average decrease in neutrophils.

Example 12. Pharmacokinetic studies of exemplary C5aR1 antibodies in hC5aR1 mice

[0358] This example shows the pharmacokinetic (PK) studies of exemplary C5aR1 antibodies in mice.

[0359] 5mg/kg of exemplary monospecific and biparatopic antibodies were injected intravenously into Tg32 mice. The amount of the antibody in the serum was evaluated over a period of 500 hours. **FIG. 16A** shows the pharmacokinetic properties of exemplary antibodies. **FIG. 16B-16D** show the stability of the anti-C5aR1 antibodies in the serum of 5 different mice for 500 hours. It was observed that monospecific (c2139 – **FIG. 16C**) and Biparatopic (c2137-e1711 – **FIG. 16D**) antibodies were stable in serum and persist equivalently in Tg32 mice. In hC5aR1 transgenic mice, TMDD observed with both c2139 and c2137-e1711. The tetravalent antibodies have slightly lower persistence in serum as compared to the monospecific antibody. Motavizumab was used as a control.

Example 13. Prediction of Pharmacokinetic studies of exemplary C5aR1 antibodies in human beings

[0360] Non-linear PK was observed for known anti-C5aR1 antibodies (Lee et al, 2006, PMID 16980984). It was reported that >90% receptor occupancy was observed for 4.3 weeks

with a 10mg/kg IV dose and >90% receptor occupancy was observed for 1.8 weeks at a 4mg/kg subcutaneous dose. Based on the affinity data of the instant humanized anti-C5aR1 antibodies, model simulations showed a non-linear clearance for between 0.1 to 10mg/kg of antibody id predicted to result in >90% receptor occupancy for 24 days, following IV administration.

[0361] Notwithstanding bioavailability, it is predicted that a 4mg/kg subcutaneous dosage is predicted to have a >90% receptor occupancy for about 7-13 days.

[0362] It was observed that the tetravalent antibodies (biparatopic) had slightly lower persistence in serum as compared to monospecific lead.

Example 14. Stability of exemplary C5aR1 antibodies at 4°C

[0363] This example shows the stability of exemplary C5aR1 antibodies (both monospecific and biparatopic) at 4°C, with one thaw cycle in between.

[0364] Both monospecific and biparatopic antibodies were incubated at 4°C for up to 14 days. After one thaw cycle at 37°C, the amount of intact antibodies were evaluated by gel filtration, native PAGE and dynamic light scattering (DLS). **Table 10** shows percent antibody aggregates (HMW), compared to percent intact antibody.

Table 10. Stability of C5aR1 bispecific antibody after 1 freeze-thaw cycle at room temperature/37°C.

Sample Information					T = 0 week		T = 1 week		T = 2 week		37c T1 day		F/T 1 cycle	
Format	**Date of Purification / Actual Time point**	**Lot #**	**Sample Name**	**Temp**	**%HMW**	**%Main**	**%HMW**	**%Main**	**%HMW**	**%Main**	**%HMW**	**%Main**	**%HMW**	**%Main**
	9/29/2020	10077	c2139	2-8C	11.7	88.3	11.2	88.8	10.7	89.4	10.0	90.1	9.7	90.3
	(1, 2, 3, 5 wk)			RT			10.6	89.4	10.1	89.9				
Tetravalent, scFv Format	8/11/2002	9996	c2137-e1711	2-8C	8.5	91.5	8.4	91.6	8.3	91.7	8.1	91.9	8.1	91.9
	(8, 9, 10, 12 wk)			RT			8.4	91.6	8.3	91.7				

[0365] It was observed that both monospecific and biparatopic antibodies were stable in 4°C and RT up to 2 weeks and one Freeze/thaw cycle.

Example 15 – Kinetic analyses of C5aR1 antagonistic humanized antibodies with modified Fc domain.

[0366] This Example describes the binding affinity and specificity of Site II monospecific antibody and biparatopic antibodies described herein, to C5aR1 and C5aR2 with modified Fc domain. The Fc domain modifications were introduced to counteract effector functions, such as ADCC or ADCP. Additionally, the modified Fc domain comprises mutation to prevent Fab arm exchange. **Table 11** summarizes Fc modifications in C5aR1 antibodies.

Table 11 – Summary of monospecific and biparatopic antibodies C5aR1 antibodies with modified Fc domains.

	MONOSPECIFIC	**BIPARATOPIC**
Name	c2139-$F_c$mod	c2137-e1711-$F_c$mod
V_H region	V_H region c2137	V_H region c2137-e1711
Fc-silencing mutations	F234V, L235E, D265G	F234V, L235E, D265G
Fab-arm exchange mutation	S228P	S228P

[0367] The binding affinity of monospecific and biparatopic antibodies with modified Fc domain were calculated as described in Example 1. The monospecific antibody with modified Fc domain is hereinafter called "c2139-$F_c$mod;" and the biparatopic antibody with modified Fc domain is hereinafter called "c2137-e1711-$F_c$mod."

(a) Binding affinity

[0368] Affinity curves of a monospecific C5aR1 antibody (c2139-Fcmod) and a C5aR1 biparatopic antibody (c2137-e1711-$F_c$mod) are illustrated in **FIG. 17A**.

[0369] It was observed that under the conditions described above, the monospecific C5aR1 monospecific antibody, c2139-Fcmod, bound C5aR1 with an affinity of about 0.31nM and the C5aR1 biparatopic antibody, c2137-e1711-$F_c$mod, bound C5aR1 with an affinity of about 0.34nM.

[0370] Exemplary kinetic assays for binding to C5aR1 expressing cells are shown in (**FIG. 17B**). Kinetic measures were fitted after a 2-3 dose association phase followed by dissociation. It was observed that EC50 of Fc modified antibodies were comparable to the EC50

of the Fc unmodified antibody. For example, as seen from **Table 12**, the EC50 of biparatopic antibody, c2137-e1711 and c2137-e1711-$F_c$mod were both about 1nM in U937-C5aR1 cells, about 1nM and 2nM in Neutrophils and 0.5nM and 0.6mM in Macrophages.

Table 12 – Comparison of EC50 of c2137-e1711 and c2137-e1711-$F_c$mod

	U937-C5aR1	**Neutrophils**	**Macrophages**
c2137-e1711-$F_c$mod	Kon: 2.6e4 (1/(M*s)) Koff: 3.4e-5 (1/s) KD: ~1 nM	Kon: 2.6e4 (1/(M*s)) Koff: 3.4e-5 (1/s) KD: ~1 nM	Kon: 8.8e4 (1/(M*s)) Koff: 3.8e-5 (1/s) KD: ~0.5 nM
c2137-e1711	Kon: 1.5e4 (1/(M*s)) Koff: 1.6e-5 (1/s) KD: ~1 nM	Kon: 6.9e3 (1/(M*s)) Koff: 2.0e-5 (1/s) KD: ~2 nM	Kon: 9.7e4 (1/(M*s)) Koff: 5.8e-5 (1/s) KD: ~0.6 nM

(b) Binding specificity

[0371] The specificity of the Fc-modified C5aR1 antagonistic antibodies was determined by measuring the affinity of anti-C5aR1 antibodies to C5aR2. This was determined as described in Example 1.

[0372] **FIG. 17C** shows the affinity curves for determining the specificity of c2137-e1711-$F_c$mod and c2139-Fcmod. It was observed that the Fc modified antibodies did not display affinity for C5aR2.

Example 16 – Internalization of C5aR1 antibodies.

[0373] The Example seeks to verify that the internalization of the C5aR1 antibodies is specific and not due to non-specific clustering of the membrane immunoglobulin. Sodium azide is a metabolic inhibitor, and inhibits internalization or endocytosis as these are energy dependent processes. Any metabolic dependency on the internalization of exemplary C5aR1 antibodies were probed using 10nM C5aR1 antibodies in the presence or absence of 20mM sodium azide. It was observed that the exemplary C5aR1 antibodies underwent metabolic-based internalization. For example, c2139 underwent internalization several hours after dissociation and c2139-e1711 undergoes internalization during association. The dissociation constant of monospecific C5aR1 antibody, c2139, in the presence of 20mM sodium azide was 1.43×10^{-5} per second, while the dissociation constant without sodium azide was 3.4×10^{-5}per second. The association constant

of biparatopic C5aR1, c2137-e1711, in the presence of 20mM sodium azide was 2.15×10^4 per mol per second, while the dissociation constant without sodium azide was 4.38×10^3per mol per second. **FIGs. 18A-18B** demonstrate increase in internalization of the C5aR1 antibodies. **FIG. 18A** is an exemplary graph showing internalization several hours after dissociation of monospecific antibody, c2139. **FIG. 18B** is an exemplary graph showing increase in internalization of biparatopic antibody, c2137-e1711, during association.

[0374] It was observed that the non-specific clustering of the membrane immunoglobulin was not solely responsible for internalization of the exemplary c5aR1 antibodies.

Example 17 – Inhibition of Gα signaling by Fc modified C5aR1 antibodies

[0375] This Example describes the functional aspect of Fc modified C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of Gα signaling using a GeneBLazer assay.

[0376] This experiment was done as detailed in Example 2. **FIGs. 19A-19B** shows the results of inhibition of Gα signaling in the presence of C5aR1 Fc-modified antibodies - c2137-e1711-$F_c$mod and c2139-$F_c$mod in comparison to Avacopan and anti-C5aR1 control Ab. It was observed that both c2137-e1711-$F_c$mod and c2139-Fcmod potently inhibited Gα signaling in a dose dependent manner.

[0377] **Table 13** summarizes the inhibition compared to avacopan and anti-C5aR1 control Ab at 10nM and 100nM concentrations of C5a. It was observed that c2137-e1711-$F_c$mod and c2139-Fcmod retained superior inhibition compared to avacopan and anti-C5aR1 control Ab even at higher concentrations of C5a.

Table 13 – Inhibition of c2137-e1711-$F_c$mod and c2139-$F_c$mod in the presence of C5a compared to avacopan and anti-C5aR1 control Ab

Antagonist	**IC50 (nM)**	
	10 nM C5a	**100 nM C5a**
c2139-Fcmod	0.09	0.05
c2137-e1711-$F_c$mod	0.06	0.04

Avacopan	2.2	0.7*
Anti-C5aR1 control Ab	4.7	6.2

*** Extrapolated data – previous data with avacopan suggests it is not very potent at high C5a concentration**

Example 18 – Inhibition of Calcium signaling by Fc modified C5aR1 antibodies

[0378] This Example describes the functional aspect of C5aR1 Fc-modified monospecific antibodies and biparatopic antibodies described herein, for inhibition of C5a mediated calcium signaling, using calcium flux assays.

[0379] This experiment was done as described in Example 3. **FIGs. 20A-20B** show the results of inhibition of Calcium signaling in the presence of C5aR1 Fc-modified antibodies - c2137-e1711-$F_c$mod and c2139-$F_c$mod in comparison to Avacopan and anti-C5aR1 control Ab in U937-C5aR1 cells. It was observed that c2137-e1711-$F_c$mod and c2139-$F_c$mod more potently inhibited calcium flux than avacopan and anti-C5aR1 control Ab. It was observed that at least 10–100× were needed to reach inhibitory levels shown by c2137-e1711-$F_c$mod and c2139-$F_c$mod. It was observed that Fc modified C5aR1 antibodies inhibited calcium signaling in human neutrophils as well as U937-C5aR1 cells. **FIG. 20A** is a dose response curve showing the exemplary graph showing inhibition of calcium signaling using an exemplary Fc modified humanized Site II antibody, as described in this disclosure, in the presence of increasing concentrations of the antibody and 100 nM C5a. **FIG. 20B** is a percent inhibition graph showing inhibition of calcium signaling using an exemplary Fc modified humanized Site II antibody, as described in this disclosure, in the presence of the antibody and 100nM C5a.

[0380] **FIGs. 21A-21D** shows the inhibition in U937-C5aR1 cells as compared to human neutrophils. **FIG. 21A** shows inhibition of calcium signaling in U937-C5aR1 cells in the presence of 10nM C5a. **FIG. 21B** shows inhibition of calcium signaling in U937-C5aR1 cells in the presence of 100nM C5a. **FIG. 21C** shows inhibition of calcium signaling in human neutrophils in the presence of 10nM C5a. **FIG. 21D** shows inhibition of calcium signaling in human neutrophils in the presence of 100nM C5a.

[0381] Further, the Fc modified antibodies were probed for inhibition of C5a mediated calcium signaling in U937-C5aR1 cells after incubation with indicated concentrations of the antagonistic antibodies at various incubation times (e.g., 1 hr and 3 hrs). **FIGs. 22A-22B** summarize the saturation levels of the U937-C5aR1 cells with each inhibitor. **FIG. 22A** summarizes percent saturation and F norm of U937-C5aR1 cells incubated with increasing concentrations of antibody, (c2139-Fcmod and c2137-e1711-Fcmod) and 100nM C5a, after 1 hour incubation. **FIG. 22B** summarizes percent saturation and F norm of U937-C5aR1 cells incubated with increasing concentrations of antibody, (c2139-Fcmod and c2137-e1711-Fcmod) and 100nM C5a, after 3 hour incubation.

[0382] **FIG. 22A** shows the inhibitory dose-response of calcium signaling by c2137-e1711-Fcmod and c2139-Fcmod. **FIG. 22B** shows the percent inhibition of calcium signaling by c2137-e1711-Fcmod and c2139-Fcmod.

[0383] It was observed that c2137-e1711-Fcmod was more potent than avacopan and anti-C5aR1 control Ab. Further, c2139-Fcmod reached a saturation point at short antagonist incubation times. This saturation is mitigated somewhat with longer antagonist incubation times. This phenomenon is only observed with c2139-Fcmod and was observed with the precursor c2139.

Example 19 – Inhibition of β-Arrestin signaling by Fc modified C5aR1 antibodies

This Example describes the functional aspect of Fc-modified C5aR1 monospecific antibody, and biparatopic antibodies described herein, for inhibition of β-arrestin recruitment.

[0384] The experimental details of this determining β-arrestin recruitment are described in Example 6. **FIGs. 23A-23B** summarize the inhibition of C5a-meditated β-arrestin signaling by c2137-e1711-Fcmod and c2139-Fcmod. It was observed that c2137-e1711-Fcmod and c2139-Fcmod more potently blocked β-arrestin recruitment to C5aR1 than avacopan and anti-C5aR1 control Ab. **Table 14** summarizes the K_D of inhibition of β-arrestin recruitment. **Table 14** – K_D of beta-arrestin inhibition

Antagonist	**IC50 (nM) at 100 nM C5a**

c2139-Fcmod	1.9
c2137-e1711-Fcmod	0.5
Avacopan	34
Anti-C5aR1 control Ab	12.7

Example 20 – Inhibition of neutrophil chemotaxis by Fc modified C5aR1 antibodies

[0385] This Example describes the functional aspect of Fc-modified C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of neutrophil chemotaxis, which is known to be induced by C5aR1 activity, using a Boyden Chamber.

[0386] The experimental details of this determining neutrophil chemotaxis are described in Example 4. **FIGs. 24A-24D** show the inhibition of chemotaxis in C5aR1-U937 stable cells after treatment with C5aR1 antibodies, c2137-e1711-Fcmod and c2139-Fcmod. It was observed that c2137-e1711-Fcmod and c2139-Fcmod more potently inhibited chemotaxis than avacopan and anti-C5aR1 control Ab. **FIG. 24A** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM c2139-Fcmod and increasing concentration of C5a. **FIG. 24B** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM C5a c2137-e1711-Fcmod and increasing concentration of C5a. **FIG. 24C-24D** shows the inhibition of chemotaxis in C5aR1-U937 stable cells in the presence of 1nM, 3.16nM and 10nM anti-C5aR1 control Ab and Avacopan, respectively, in the presence of c2137-e1711-Fcmod.

Example 21 – Inhibition of CD11b expression by Fc modified C5aR1 antibodies

[0387] This Example describes the functional aspect of Fc-modified C5aR1 monospecific antibodies and biparatopic antibodies described herein, for inhibition of CD11b expression.

[0388] The experimental details of this determining CD11 expression are described in Example 5. **FIGs. 25A-25B** show the inhibition of CD11b signaling in response to treatment with c2137-e1711-Fcmod and c2139-Fcmod. It was observed that c2137-e1711-Fcmod and c2139-Fcmod potently inhibited CD11b expression across the wide range of C5a concentrations, compared to avacopan and anti-C5aR1 control Ab. **FIG. 25A** shows the inhibition of CD11b signaling in the presence of increasing concentration of C5aR1 antagonistic antibody and 10nM C5a. **FIG. 25B** shows the inhibition of CD11b signaling in the presence of increasing concentration of C5aR1 antagonistic antibody and 100nM C5a.

Example 22 – Inhibition of ROS production by Fc modified C5aR1 antibodies

[0389] This example shows that exemplary humanized Fc-modified monospecific antibodies (c2139-Fcmod) and exemplary biparatopic antibodies (c2137-e1711-Fcmod) reduced reactive oxygen species (ROS).

[0390] The experimental details of this determining ROS production are described in Example 7. **FIGs. 26A-26B** shows the inhibition of ROS production in The RBC-lysed WB cells treated with c2139-Fcmod, c2137-e1711-Fcmod, compared to c2139, c2137-e1711, anti-C5aR1 control Ab and avacopan. It was observed that c2139-Fcmod, and c2137-e1711-Fcmod maintained low respiratory burst activity in neutrophils, similar to avacopan and anti-C5aR1 control Ab. Further, c2139-Fcmod, and c2137-e1711-Fcmod had reduced respiratory burst activity compared to previous forms c2139, and c2137-e1711. **FIG. 26A** shows inhibition of ROS production of increasing concentration of monospecific C5aR1 antibody (Fc modified). **FIG. 26B** shows inhibition of ROS production of increasing concentration of biparatopic C5aR1 antibody (Fc modified).

Example 23. Inhibition of Neutropenia in human C5aR1 transgenic mice by Fc modified C5aR1 antibodies

[0391] This example shows the inhibition of neutropenia by Fc-modified C5aR1 antibodies in transgenic human C5aR1 mice. As described above (Example 11), the exemplary C5aR1 antibodies do not cross-react with mouse C5aR1. Transgenic human C5aR1 (hC5aR1) knock-in mice were generated using CRISPR technology at The Jackson Laboratory.

[0392] **FIG. 27A** shows the experiment design in mice to evaluate alleviation of neutropenia. The experimental details of this mouse *in vivo* assay are described in Example 11. The percent chance in neutrophils and average change in neutrophils were calculated in all groups and are shown in **FIG. 27B-27C**. The average change in neutrophils are shown in **FIG. 27B**. Change in neutrophil counts are shown in **FIG. 27C**. It was observed that the new Fc-silenced leads were just as potent as non-silenced c2139, and c2137-e1711. The vehicle (PBS) group demonstrated robust neutropenia with 65% average reduction in neutrophil counts from baseline at 1 min post-C5a injection. One animal did not respond to C5a in the Vehicle control group.

Example 24. Protection of Stroke in a mouse model

[0393] This Experiment demonstrated the ability of c2139-$F_c$mod, and c2137-e1711-$F_c$mod to protect infract volume in a stroke mouse model. Neutrophil activity and infiltration to the brain are well documented in stroke and traumatic brain injury pathology, including in acute models.

[0394] 20mg/kg of each, c2139-$F_c$mod, and c2137-e1711-$F_c$mod, were administered in tMCAO mice. 1mg/kg PMX53 was used as a control. The brain was resected, cut into slices and stained with TTC. The stained cross sections were analyzed by imaging.

[0395] It was observed that mice treated with c2139-$F_c$mod, and c2137-e1711-$F_c$mod provided protection against stroke by significantly reducing the infarct volume, compared to the vehicle. c2137-e1711-$F_c$mod treatment showed significantly reduced infarct volume compared to the vehicle group. c2139-$F_c$mod treatment showed reduced infarct volume compared to the vehicle group. PMX53 treatment (serving as a positive control and comparator) showed only slightly reduced infarct volume compared to the vehicle group. **FIG. 28A** shows a pictorial representation of the reduction in the infract volume. **FIG. 28B** shows a graphical representation of the infarct volume after treatment with c2139-$F_c$mod, c2137-e1711-$F_c$mod and PMX53.

Example 25. Pharmacokinetic studies of exemplary C5aR1 antibodies in hC5aR1 mice with modified Fc domains

[0396] This example shows the pharmacokinetic (PK) studies of exemplary C5aR1 with modified Fc domains antibodies in mice. Tg32 mice are a transgenic model in which human FcRn replaces native mouse FcRn. FcRn is necessary for the bidirectional transport of Abs across cellular barriers, influencing PK. Tg32 mice with human FcRn have been extensively studied and thought to correlate with PK in humans. The goal of this experiment was to assess FcRn-mediated recycling of IgG-scFv vs IgG formats and assess any impact of introducing Fc domain modifications on FcRn mediated recycling.

[0397] 5mg/kg of exemplary monospecific and biparatopic antibodies were injected intravenously into Tg32 mice. The percentage of antibody in the serum was determined using art-recognized assays. MVZ-IgG4-VFc17, a motavizumab antibody with IgG4 Fc and same Fc modifications as the C5aR1 antibodies, was used as a control. Further PK/PD analyses were carried out using CERTARA.

[0398] It was observed that C-max (the highest concentration of the antibodies in blood was similar for both monospecific and biparatopic antibodies). Further, it was observed that the half-life of the antibodies were similar for c2139-$F_c$mod, and c2137-e1711-$F_c$mod.

[0399] The amount of the antibody in the serum was evaluated over a period of 500 hours. **Table 15** shows the dosage and the sampling intervals for the pharmacokinetic studies.

Table 15 – Dosing and sampling schedule for exemplary C5aR1 Fc modified antibodies to determine the pharmacokinetics for individual antibodies.

Antibody	**Dose (mg/Kg)**	**N**	**Blood sampling**
c2139-$F_c$mod	5	5	1h, 8h, 1d, 2d, 3d, 4d, 6d, 8d, 10d, 12d, 15d, 18d, 21d
c2137-e1711-$F_c$mod	5	5	
MVZ-IgG4-VFc17	5	5	

[0400] **FIGs. 29A-29B** shows the pharmacokinetic properties of C5aR1-Fc modified antibodies. **FIG. 29A** is a graphical representation of the percentage of the Fc modified C5aR1 antibodies in the blood serum for 500 hours. **FIG. 29B** is a graphical representation of mean concentration in µg antibody/ml of serum over 500 hours. These observations showed that c2137-e1711-$F_c$mod efficiently engaged with hFcRn and recycled in a manner similar to c2139-

Fcmod. Further, the PK data were also similar to the PK of C5aR1 antibodies with unmodified Fc domains. **Table 16** shows the PK parameters for the exemplar Fc-modified C5aR1 antibodies.

Table 16 – Pharmacokinetic parameters of Fc modified C5aR1 antibodies.

		Parameter							
		C_{max} (µg/ml)	t_{last} (h)	AUC_{0-24} (µg*h/ml)	AUC_{last} (µg*h/ml)	AUC_{inf} (µg*h/ml)	$t_{1/2}$ (h)	CL (ml/h/kg)	V (ml/kg)
Ab	**Dose**	**GeoMean (Geo CV%)**							
c2139-Fcmod	5 mg/kg	52.6 (29.1%)	504 (504 - 504)*	904 (26.4%)	9230 (8.3%)	11100 (17.3%)	229 (89.2 - 242)*	0.449 (17.3%)	118 (38.2%)
c2137-e1711-Fcmod	5 mg/kg	40.8 (30.1%)	504 (504 - 504)*	699 (18%)	7550 (23.2%)	9500 (38.9%)	221 (67.1 - 390)*	0.526 (38.9%)	135 (45.1%)
Isotype CTL	5 mg/kg	131 (29.2%)	504 (504 - 504)*	1620 (7.7%)	13100 (7.5%)	16900 (10.4%)	259 (200 - 270)*	0.295 (10.4%)	104 (7.4%)
*Value presented as Median (Min - Max)									

Example 26 - Multi-dose pharmacokinetics of Fc-modified C5aR1 antibodies in human C5aR1 Knock-in Mice

[0401] This example shows the multi-dose pharmacokinetic (PK) studies of exemplary C5aR1 antibodies with modified Fc domains antibodies hC5aR1 mice.

[0402] **Table 17** summarizes the dosage regimen of the antibodies being tested. Target-Mediated Drug Disposition (TMDD) is evident for both exemplary C5aR1 antibodies with modified Fc domains. TMDD is the phenomenon in which a drug binds with high affinity to its pharmacological target site (such as a receptor) to such an extent that this affects its pharmacokinetic (PK) characteristics. The target binding and subsequent elimination of the drug-target complexes could affect both drug distribution and elimination, and result in nonlinearity of PK in a dose-dependent manner.

[0403] TMDD is most observed as linear PK at high dose levels and nonlinear PK at low dose levels

[0404] Dose-dependent clearance was observed as is expected for an antibody with TMDD. At 5mg/Kg, and 0.5mg/Kg, maximum concentration of each antibody, C_{max} was found to be similar for both antibodies. Further, at 5mg/Kg, and 0.5mg/Kg, half-life of each antibody, ($T_{0.5}$), was found to be similar. At 20mg/kg, c2139 had a better half-life, ($T_{0.5}$), than c2137-e1711.

Table 17 – Dosing and sampling schedule for exemplary C5aR1 Fc modified antibodies to determine the pharmacokinetics for individual antibodies.

Group	Antibody	Dose (mg/Kg)	N	Blood sampling
1	c2139-Fcmod	0.5	4	1h, 8h, 1d, 2d, 3d, 4d, 6d, 8d, 10d, 12d, 15d, 18d, 21d
2	c2139-Fcmod	5	4	
3	c2139-Fcmod	20	4	
4	c2137-e1711-Fcmod	0.5	4	
5	c2137-e1711-Fcmod	5	4	
6	c2137-e1711-Fcmod	20	4	
7	MVZ-IgG4-VFc17	20	4	

[0405] The target binding and subsequent elimination of the drug-target complexes could affect both drug distribution and elimination, and result in nonlinearity of PK in a dose-dependent manner. **FIG. 30A-30F** show antibody persistence was dose-dependent. **Table 18** shows the PK parameters for the exemplar Fc-modified C5aR1 antibodies. **FIG. 30A** is a graphical representation of a dose response curve of c2139-Fcmod in serum for 200 hours. **FIG. 30B** is a graphical representation of a dose response curve of c2137-e1711-Fcmod in serum for 200 hours. **FIG. 30C** is a comparison of c2139Fcmod, c2137-e1711-Fcmod and MVZ-IgG4. **FIG. 30D** is *in silico* graphical representation of c2139 at three different concentrations over a period of 500 hours. **FIG. 30E** is an *in silico* graphical representation of c2137-e1711 at three different concentrations over a period of 500 hours. **FIG. 30F** is *in silico* graphical representation of isotype control antibody over a period of 500 hours at a concentration of 20mg/Kg. Non-naïve mice were administered multiple dosing of the mAbs once weekly over 4 total weeks. It was observed that both c2139Fcmod, c2137-e1711-Fcmod maintain exposure with once weekly IV dosing at 5 mg/kg. No severe outcomes were observed with mAbs dosed once weekly for several weeks.

PK parameters for the exemplar Fc-modified C5aR1 antibodies.

C_{max} (µg/ml)	t_{last} (h)	AUC_{0-24} (µg*h/ml)	AUC_{last} (µg*h/ml)	AUC_{inf} (µg*h/ml)	$t_{1/2}$ (h)	CL (ml/h/kg)	V (ml/kg)
GeoMean (Geo CV%)							
355 (12.2%)	504 (504 - 504)*	4880 (12%)	34200 (20.8%)	36700 (26.2%)	135 (105 - 163)*	0.545 (26.2%)	105 (14.2%)
510 (34.6%)	396 (288 - 504)*	6750 (23.2%)	30100 (28.9%)	30400 (29.8%)	31.6 (16.1 - 90.0)*	0.657 (29.8%)	32.6 (65.5%)
453 (29.4%)	504 (288 - 504)*	7130 (22.1%)	55800 (23.4%)	66200 (20.8%)	127 (116 - 192)*	0.302 (20.8%)	60.0 (26.7%)
87.9 (23.7%)	264 (192 - 432)*	1180 (19.6%)	3680 (34%)	3780 (34.8%)	51.6 (24.7 - 72.0)*	1.32 (34.8%)	89.0 (27.1%)
84.3 (30%)	216 (144 - 288)*	1140 (10.3%)	3590 (15.4%)	4080 (23.1%)	49.7 (48.5 - 110)*	1.22 (23.1%)	113 (24.8%)
8.45 (33.4%)	24.0 (24.0 - 24.0)*	90.7 (19.6%)	90.7 (19.6%)	114 (20.7%)	10.5 (7.62 - 11.4)*	4.40 (20.7%)	62.8 (25.4%)
8.05 (21%)	24.0 (24.0 - 24.0)*	89.1 (10.2%)	89.1 (10.2%)	119 (9.5%)	11.7 (9.13 - 12.9)*	4.21 (9.5%)	68.4 (16.9%)
(Min - Max)							

Example 27 – Inhibition of Neutropenia in Human C5aR1 KI Mice, by Fc-modified C5aR1 antibodies at low dose

[0407] This example shows that c2139-Fcmod and c2137-e1711-Fcmod can inhibit neutropenia in Human C5aR1 knock in mice at low doses.

[0408] **Table 19** summarizes the dosage regimen of the antibodies being tested, and the bleed times to determine neutropenia. Neutrophil change from baseline (-5 min bleed) at 1 min post C5a administration are shown in **FIG. 31**.

[0409] It was observed that c2139-Fcmod and c2137-e1711-Fcmod potently inhibited neutropenia induced by C5a administration compared to vehicle control, even at 0.1 mg/kg doses.

Table 19. Dosage Regimen

Test article	**# mice**	**dose**	**Neutropenia bleeds**				
			-5min	***0hr***	***1 min***	***5min***	***2hr***
c2137-e1711-Fcmod	5	0.01 mg/kg IV	blood	0.1 mg/kg hC5a IV	blood	blood	blood
c2137-e1711-Fcmod	5	0.1 mg/kg IV					
c2137-e1711-Fcmod	5	0.5 mg/kg IV					
c2137-e1711-Fcmod	1	3 mg/kg IV					
c2139-Fcmod	5	0.01 mg/kg IV					
c2139-Fcmod	5	0.1 mg/kg IV					
c2139-Fcmod	5	0.5 mg/kg IV					
c2139-Fcmod	1	3 mg/kg IV					
Isotype mAb CTL	5	0.5 mg/kg IV					
Vehicle (PBS)	5	n/a					

Example 28 – Internalization of Fc modified C5aR1 antibodies

[0410] This example shows the internalization of Fc modified humanized monospecific and biparatopic C5aR1 antibodies in hC5ar1-U937 cells.

[0411] The Fc modified, humanized monospecific anti-C5aR1 antibodies, c2137-e1711-Fcmod and c2139-Fcmod were conjugated to pH sensitive dye (DyLight488), which fluoresces brightly at low pH but is non-fluorescent at neutral pH. The conjugated antibodies were incubated with U937 cells and hC5aR1 knock in U937 cells. **FIG. 32A** shows the fluorescence intensity after 6 hours and 24 hours of incubation with each conjugated antibody.

[0412] It was observed that the exemplary Fc modified C5aR1 antibodies underwent metabolic-based internalization. **FIG. 32B** shows the internalization of both c2137-e1711-Fcmod and c2139-Fcmod in live cells as observed by Nikon confocal experiment over a period of 300 min.

EQUIVALENTS AND SCOPE

[0413] Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the disclosure described herein. The scope of the present disclosure is not intended to be limited to the above description, but rather is as set forth in the following claims:

CLAIMS

1. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a heavy chain variable region (VH), wherein the VH comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 14.

2. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a light chain variable region (VL), wherein the VL comprises an amino acid sequence with at least 90% identity to, the amino acid sequence of SEQ ID NO: 25.

3. An antibody or antigen binding fragment thereof, comprising a VH region, wherein the VH comprises three heavy chain complementarity determining regions (HCDRs), wherein the HCDR1, HCDR2 and HCDR3 sequences comprising amino acid sequences of SEQ ID Nos: 6 (NYWMH), 7 (YLNPSSGYTKYAQKFQG) and 8 (SGGDNYGNPYYFDR), respectively.

4. An antibody or antigen binding fragment thereof, comprising a VL region, wherein the VL comprises three light chain complementarity determining regions (LCDRs), wherein the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID Nos: 9 (RASQSIVHSNGNTYLH), 10 (KVSNRFS) and 11 (AQYTLVPLT), respectively.

5. An antibody or antigen binding fragment thereof, comprising a VH region, wherein the VH comprises three heavy chain complementarity determining regions (HCDRs), wherein the HCDR1, HCDR2 and HCDR3 sequences comprising amino acid sequences of SEQ ID Nos: 6 (NYWMH), 7 (YLNPSSGYTKYAQKFQG) and 8 (SGGDNYGNPYYFDR), respectively and a VL region, wherein the VL comprises three light chain complementarity determining regions (LCDRs), wherein the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID Nos: 9 (RASQSIVHSNGNTYLH), 10 (KVSNRFS) and 11 (AQYTLVPLT), respectively.

6. The antibody or antibody or antigen binding fragment thereof according to any of claims 1-5, wherein the antibody or antigen binding fragment thereof, further comprises a Fc region.

7. The antibody or antibody or antigen binding fragment thereof according to any of claims 1-6, wherein the Fc domain is independently selected from IgG1, IgG2, IgG3, and IgG4.

8. The antibody or antigen binding fragment thereof of any one of claims 1-7, wherein the antibody binding to C5aR1 inhibits interaction of complement component 5a (C5a) with C5aR1.

9. The antibody or antigen binding fragment thereof of any one of claims 1-8, wherein the antibody does not bind to C5aR2.

10. The antibody or antigen binding fragment thereof of any one of claims 1-9, wherein the antibody or antigen binding fragment is humanized.

11. The antibody or the antigen binding fragment thereof, according to any one of the previous claims wherein the VH or the VL has been modified to enhance the stability of the molecule.

12. The antibody or the antigen binding fragment thereof, according to claim 11, wherein the VL comprises a serine or tyrosine at position 96 of SEQ ID NO: 5 or SEQ ID NO: 25.

13. The antibody or antigen binding fragment thereof of any one of claims 1-12, wherein the antibody or antigen binding fragment does not cross-react with mouse C5aR1.

14. The antibody or antigen binding fragment thereof of any one of claims 1-13, wherein the antibody binds C5aR1 at an affinity of between 10pM to 50nM.

15. The antibody or antigen binding fragment thereof of any one of claims 1-14, wherein the antibody binds C5aR1 at an affinity of 0.16nM or lower.

16. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1 inhibits neutrophil chemotaxis.

17. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a concentration of at least 10nM.

18. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1 inhibits C5a mediated C5aR1 Gα signaling.

19. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1inhibits calcium signaling.

20. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1 inhibits CD11b expression.

21. The antibody or antigen binding fragment thereof of any one of claims 1-15, wherein the antibody binding to C5aR1 inhibits neutropenia.

22. The antibody or antigen binding fragment thereof of any of claims 1-15, wherein the antibody binding to C5aR1 inhibits β-arrestin signaling.

23. The antibody or antigen binding fragment thereof of any of claims 1-15, wherein the antibody binding to C5aR1 inhibits ROS production in neutrophils.

24. The antibody or antigen binding fragment thereof of any one of the previous, wherein the antibody is stable at 4°C for up-to 2 weeks with one freeze thaw cycle.

25. A nucleic acid encoding an antibody or antigen binding fragment thereof of claim any one of previous claims.

26. A cell comprising the nucleic acid of claim 23.

27. A method of making an antibody or antigen binding fragment thereof of claim any one of the previous claims method comprising; culturing a host cell comprising a nucleic acid encoding the antibody or antigen binding fragment thereof, an culturing the cell under conditions that allow production of the antibody or antigen binding fragment thereof.

28. A method of treating an autoimmune disease, comprising administering an antibody or an antigen binding fragment thereof to a subject in need of the treatment, wherein the antibody or the antigen binding fragment thereof binds complement component 5a receptor 1 (C5aR1) and comprises a heavy chain variable region (VH) having an amino acid sequence with at least 90% identity to SEQ ID NO: 14 and a light chain variable region (VL) having an amino acid sequence with at least 90% identity to SEQ ID NO: 25.

29. Amethod of treating an autoimmune disease, comprising administering an antibody or an antigen binding fragment thereof to a subject in need of the treatment, wherein the antibody or the antigen binding fragment thereof binds human complement component 5a receptor 1 (C5aR1), the antibody or antigen binding fragment thereof comprising a heavy chain variable region (VH), wherein the VH comprises at least 90% identity to, SEQ ID NO: 14 and an antibody or antigen binding fragment thereof, that binds human complement component 5a receptor 1 (C5aR1) comprising a light chain variable region (VL), wherein the VL at least 90% identity to, SEQ ID NO: 15.

30. A method of treating an autoimmune disease, comprising administering an antibody or an antigen binding fragment thereof to a subject in need of the treatment, wherein the antibody or the antigen binding fragment thereof binds complement component 5a receptor 1 (C5aR1) and comprises:

a heavy chain comprising a HCDR1 comprising SEQ ID NO: 6 (NYWMH), a HCDR2 comprising SEQ ID NO: 7 (YLNPSSGYTKYAQKFQG), and a HCDR3 comprising SEQ ID NO: 8 (SGGDNYGNPYYFDR); and

a light chain comprising a LCDR1 comprising SEQ ID NO: 9 (RASQSIVHSNGNTYLH), a LCDR2 comprising SEQ ID NO: 10 (KVSNRFS), and a LCDR3 comprising SEQ ID NO: 11 (AQYTLVPLT).

31. A method of treating an autoimmune diseases comprising administering an antibody or an antigen binding fragment thereof to a subject in need of the treatment, wherein the antibody or the antigen binding fragment thereof comprises a heavy chain of SEQ ID NO: 4 or an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 4.

32. A method of treating an autoimmune diseases comprising administering an antibody or an antigen binding fragment thereof to a subject in need of the treatment, wherein the antibody or the antigen binding fragment thereof comprises a light chain of SEQ ID NO: 5 or an amino acid sequence with at least 85% identity to amino acid sequence of SEQ ID NO: 5.

33. A method of treating a disease caused by neutropenia comprising administering to a subject in need of the treatment an antibody or an antibody binding fragment thereof of any one of claims 1-24.

34. The method of any one of claims 33, wherein the neutropenia is caused by high levels of C5a.

35. The method of any one of claims 28-33, wherein the disease is ANCA vasculitis or lupus.

36. The method of any one of claims 28-33, wherein the disorder is rheumatoid arthritis.

37. The method of any one of claims 28-33, wherein the disorder is a kidney disorder.

38. A method of inhibition of C5a signaling using an monoclonal antibody which binds to C5aR1, wherein the monoclaonal antibody comprises:

a heavy chain variable region (VH) comprising an amino acid sequence with at least 90% identity to SEQ ID NO: 14; and

a light chain variable region (VL) comprising an amino acid sequence with at least 90% identity SEQ ID NO: 25.

39. A biparatopic antibody or antigen binding fragment thereof, comprising a first antigen binding domain comprising a VH1 and a VL1 that bind C5aR1 at SEQ ID NO: 3 and a second antigen binding domain comprising a VH2 and a VL2 that bind C5aR1 at SEQ ID NO: 1 or SEQ ID NO: 2.

40. A biparatopic antibody or antigen binding fragment thereof, comprising a first antigen binding domain comprising a VH1 and a VL1that bind C5aR1 at SEQ ID NO: 1 or SEQ ID NO: 2 and a second antigen binding domain comprising a VH2 and a VL2that bind C5aR1 at SEQ ID NO: 3.

41. The biparatopic antibody or antigen binding fragment thereof of claim 39 or claim 40, wherein the VH1 comprises an amino acid sequence of SEQ ID NO: 14 or is at least 90% identical to of SEQ ID NO: 14.

42. The biparatopic antibody or antigen binding fragment thereof of claim 39 or claim 40, wherein the VL1 comprises an amino acid sequence of SEQ ID NO: 15, or is at least 90% identical to of SEQ ID NO: 15.

43. The biparatopic antibody or antigen binding fragment thereof of claim 39 or claim 40, wherein the VH2 comprises an amino acid sequence of SEQ ID NO: 16, or is at least 90% identical to of SEQ ID NO: 16.

44. The biparatopic antibody or antigen binding fragment thereof of claim 39 or claim 40, wherein the, VL2 comprises an amino acid sequence of SEQ ID NO: 17, or is at least 90% identical to the amino acid sequence of SEQ ID NO: 17.

45. A biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 85% identical to the amino acid sequence of SEQ ID NO: 12.

46. A biparatopic antibody or antigen binding fragment thereof, comprising a light chain of SEQ ID NO: 13, or at least 85% identical to the amino acid sequence of SEQ ID NO: 13.

47. A biparatopic antibody or antigen binding fragment thereof, comprising a heavy chain of SEQ ID NO: 12, or at least 85% identical to the amino acid sequence of SEQ ID NO: 12 further comprising a light chain of SEQ ID NO: 13, or at least 85% identical to the amino acid sequence of SEQ ID NO: 13.

48. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-47, wherein the heavy chain comprises the VH1 linked to an Fc domain.

49. The biparatopic antibody or antigen binding fragment thereof of claim 48 wherein the Fc domain is further linked to a scFv comprising a VH2 comprising an amino acid sequence of SEQ ID NO: 16, or an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 16 and/or a VL2 comprising an amino acid sequence of SEQ ID NO: 17, or an amino acid sequence at least 90% identical to the amino acid sequence of SEQ ID NO: 17.

50. The biparatopic antibody or antibody or antigen binding fragment thereof of claim 48 or claim 49, wherein the Fc domain is independently selected from IgG1, IgG2, IgG3, and IgG4.

51. The biparatopic antibody or antibody or antigen binding fragment thereof of claim 49, wherein the scFv is linked to the Fc domain via a linker.

52. The biparatopic antibody of claim 51, wherein the linker comprises at least 5 amino acids comprising an amino acid sequence with any of SEQ ID NOs: 26-37.

53. The biparatopic antibody of claim 49, wherein the VH2 comprising SEQ ID NO: 16 and the VL2 comprising SEQ ID NO: 17 are linked to each other via a linker.

54. The biparatopic antibody of claim 53, wherein the linker comprises 1-10 repeats of SEQ ID NO: 31.

55. The biparatopic antibody of claim 49, wherein the VH2 and the VL2 further comprise

one or more mutations to improve thermal stability of the biparatopic antibody.

56. The biparatopic antibody or antigen binding fragment thereof of claim 55, wherein the mutation comprises incorporation of cysteine at position 559 of SEQ ID NO: 12 and at position 630 of SEQ ID NO: 12.

57. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-56, wherein the antibody binding to C5aR1 inhibits interaction of complement component 5a (C5a) with human C5aR1.

58. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-57, wherein the antibody does not bind to C5aR2.

59. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-58, wherein the antibody or antigen binding fragment is humanized.

60. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-59, wherein the antibody or antigen binding fragment does not cross-react with mouse C5aR1.

61. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-60, wherein the antibody is stable at 4°C for up-to 2 weeks with one freeze thaw cycle.

62. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-61, wherein the antibody binding to C5aR1 inhibits neutrophil chemotaxis.

63. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-62, wherein the antibody binding to C5aR1 inhibits neutrophil chemotaxis in the presence of high C5a concentration.

64. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-62, wherein the antibody binding to C5aR1 inhibits neutrophil chemotaxis in the presence of C5a

concentration of at least 10nM.

65. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-64, wherein the antibody binding to C5aR1 inhibits C5a mediated C5aR1 Gα signaling.

66. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-65, wherein the antibody binding to C5aR1inhibits calcium signaling.

67. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-66, wherein the antibody binding to C5aR1 inhibits CD11b expression.

68. The biparatopic antibody or antigen binding fragment thereof of any one of claims 39-67, wherein the antibody binding to C5aR1 inhibits neutropenia.

69. The biparatopic antibody or antigen binding fragment thereof of any of claims 39-61, wherein the antibody binding to C5aR1 inhibits β-arrestin signaling.

70. The biparatopic antibody or antigen binding fragment thereof of any of claims 39-61, wherein the antibody binding to C5aR1 inhibits ROS production in neutrophils.

71. A nucleic acid encoding the biparatopic antibody of claim any one of claims 39-68.

72. A cell comprising the nucleic acid of claim 71.

73. A method of making a biparatopic antibody or antigen binding fragment thereof of claim any one claims 39-70, the method comprising culturing a host cell comprising a nucleic acid encoding the antibody or antigen binding fragment thereof, an culturing the cell under conditions that allow production of the antibody or antigen binding fragment thereof.

74. A method of treating an autoimmune diseases using a biparatopic antibody wherein the antibody binds human complement component 5a receptor 1 (C5aR1), the comprising:

a. a first VH and a first VL, VH1 and VL1, wherein the VH1 comprises an amino acid sequence with at least 90% identity to SEQ ID NO: 14 and wherein the VL1 comprises an amino acid sequence with at least 90% identity to SEQ ID NO: 15 and

b. a second VH and a second VL, VH2 and VL2, wherein the VH2 comprises an amino acid sequence with at least 90% identity to SEQ ID NO: 16 and VL2 comprises an amino acid sequence with at least 90% identity to SEQ ID NO: 17.

75. The method of treating a disease caused by neutropenia using an antibody or an antigen binding fragment thereof according to the biparatopic antibody of any of preceding claims.

76. The method of any one of claims 73-75, wherein the neutropenia is caused by high levels of C5a.

77. The method of any one of claims 73-76, wherein the disease is ANCA vasculitis or lupus.

78. The method of any of claims 73-77, wherein the disorder is rheumatoid arthritis.

79. The method of any of claims 73-78, wherein the disorder is a kidney disorder.

80. The method of any of claims 73-78 or 27-33, wherein the disorder is stroke.

81. A monospecific or biparatopic C5aR1 antibody of any of the preceding claims, wherein the antibody comprises a modified IgG1, IgG4 or IgG2 constant domains.

82. The antibody of claim 81, wherein the C5aR1 comprises a modified IgG4 Fc domain.

83. The antibody of claim 82, wherein the modified IgG4 Fc domain comprises substitutions at positions F234, L235 and/or D265.

84. The antibody of claim 83, wherein the IgG4 Fc substitution at position F234 is a

hydrophobic amino acid selected from Alanine, Valine, Leucine, Isoleucine, phenylalanine, or tryptophan.

85. The antibody of claim 83, wherein the IgG4 Fc substitution at position F234 is a valine.

86. The antibody of claim 83, wherein the IgG4 Fc substitution at position L235 is an acidic amino acid.

87. The antibody of claim 86, wherein the IgG4 Fc substitution at position L235 is an acidic amino acid selected from glutamate or aspartate.

88. The antibody of claim 86, wherein the IgG4 Fc substitution at position L235 is aspartate.

89. The antibody of claim 83, wherein the IgG4 Fc substitution at position D265 is a non-polar amino acid.

90. The antibody of claim 89, wherein the IgG4 Fc substitution at position D265 is a non-polar amino acid selected from alanine, cysteine, glycine, isoleucine, leucine, methionine, and valine.

91. The antibody of claim 89, wherein the IgG4 Fc substitution at position D265 is glycine.

92. The antibody of any of the preceding claims, wherein the antibody further comprises a substitution at S228.

93. The antibody of claim 92, wherein the substitution at S228 is a proline.

94. A monospecific or biparatopic C5aR1 antibody of any of the preceding claims, wherein the antibody comprises a modified IgG4 constant domain comprising combination of F234V, L235E and D265G substitutions.

95. A method of reducing or preventing antibody dependent cytotoxicity, antibody dependent phagocytosis and/or Complement Dependent Cytotoxicity, using a monospecific or biparatopic C5aR1 antibody of any of claims 81-94.

96. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising:

a heavy chain variable region (VH) of SEQ ID NO: 14;

a light chain variable region (VL) of SEQ ID NO: 25; and

a modified Fc domain comprising substitutions F234V, L235E, and D265G.

97. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising:

a heavy chain variable region (VH) comprising HCDR1 of SEQ ID NO: 6 (NYWMH), a HCDR2 of SEQ ID NO: 7 (YLNPSSGYTKYAQKFQG), and a HCDR3 of SEQ ID NO: 8 (SGGDNYGNPYYFDR);

a light chain variable region (VL) comprising LCDR1 of SEQ ID NO: 9 (RASQSIVHSNGNTYLH), LCDR2 of SEQ ID NO: 10 (KVSNRFS), and LCDR3 of SEQ ID NO: 11 (AQYTLVPLT); and

a modified Fc domain comprising substitutions F234V, L235E, and D265G.

98. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising a heavy chain of SEQ ID NO: 69 and a light chain of SEQ ID NO: 70.

99. A biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising:

a light chain comprising a LCDR1 of SEQ ID NO: 9 (RASQSIVHSNGNTYLH), a LCDR2 of SEQ ID NO: 10 (KVSNRFS), and a LCDR3 of SEQ ID NO: 21 (AQSTLVPLT); and

a heavy chain comprising: a HCDR1 of SEQ ID NO: 6 (NYWMH), a HCDR2 of SEQ ID NO: 7 (YLNPSSGYTKYAQKFQG), and a HCDR3 of SEQ ID NO: 8 (SGGDNYGNPYYFDR);

a modified Fc domain comprising substitutions F234V, L235E, and D265G; and

a scFv comprising a LCDR4 of SEQ ID NO: 22 (RSSQSLVHSNGNTYLN), a LCDR5 of SEQ ID NO: 23 (KVSNRLS), a LCDR6 of SEQ ID NO: 24 (SQSTHVPYT), a HCDR4 of SEQ ID NO: 18 (AYAMS), a HCDR5 of SEQ ID NO: 19 (SISTGGNTYYADSVKG), and a HCDR6 of SEQ ID NO:20 (GYQRFSGFAY),

wherein the scFv is linked to the modified Fc domain.

100. A biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising:

a first light chain variable region (VL) of SEQ ID NO: 15;

a first heavy chain variable region (VH) of SEQ ID NO: 14;

a modified Fc domain comprising substitutions F234V, L235E, and D265G; and

a scFv comprising a second light chain variable region (VL) of SEQ ID NO: 17 and a second heavy chain variable region (VH) of SEQ ID NO of SEQ ID NO: 16,

wherein the scFv is linked to the modified Fc domain.

101. A biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising a light chain of SEQ ID NO: 72 and a heavy chain of SEQ ID NO:71.

102. A biparatopic antibody or an antigen binding fragment thereof, that binds complement component 5a receptor (C5aR1) comprising:

a first light chain variable region (VL) of SEQ ID NO: 25;

a first heavy chain variable region (VH) of SEQ ID NO: 14; and

a scFv comprising a second light chain variable region (VL) of SEQ ID NO: 17 and a second heavy chain variable region (VH) of SEQ ID NO of SEQ ID NO: 16,

wherein the scFv is linked to an Fc domain.

103. The biparatopic antibody or an antigen binding fragment thereof of claim 102, further comprising a modified Fc domain comprising substitutions F234V, L235E, and D265G, such that the scFv is linked to the modified Fc domain.

104. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising:

a heavy chain variable region (VH) of SEQ ID NO: 43; and

a light chain variable region (VL) of SEQ ID NO: 48.

105. The antibody of claim 104, further comprising a modified Fc domain comprising substitutions F234V, L235E, and D265G.

106. An antibody or antigen binding fragment thereof, that binds complement component 5a receptor 1 (C5aR1) comprising:

a heavy chain variable region (VH) of SEQ ID NO: 14; and

a light chain variable region (VL) of SEQ ID NO: 15.

107. The antibody of claim 106, further comprising a modified Fc domain comprising substitutions F234V, L235E, and D265G.

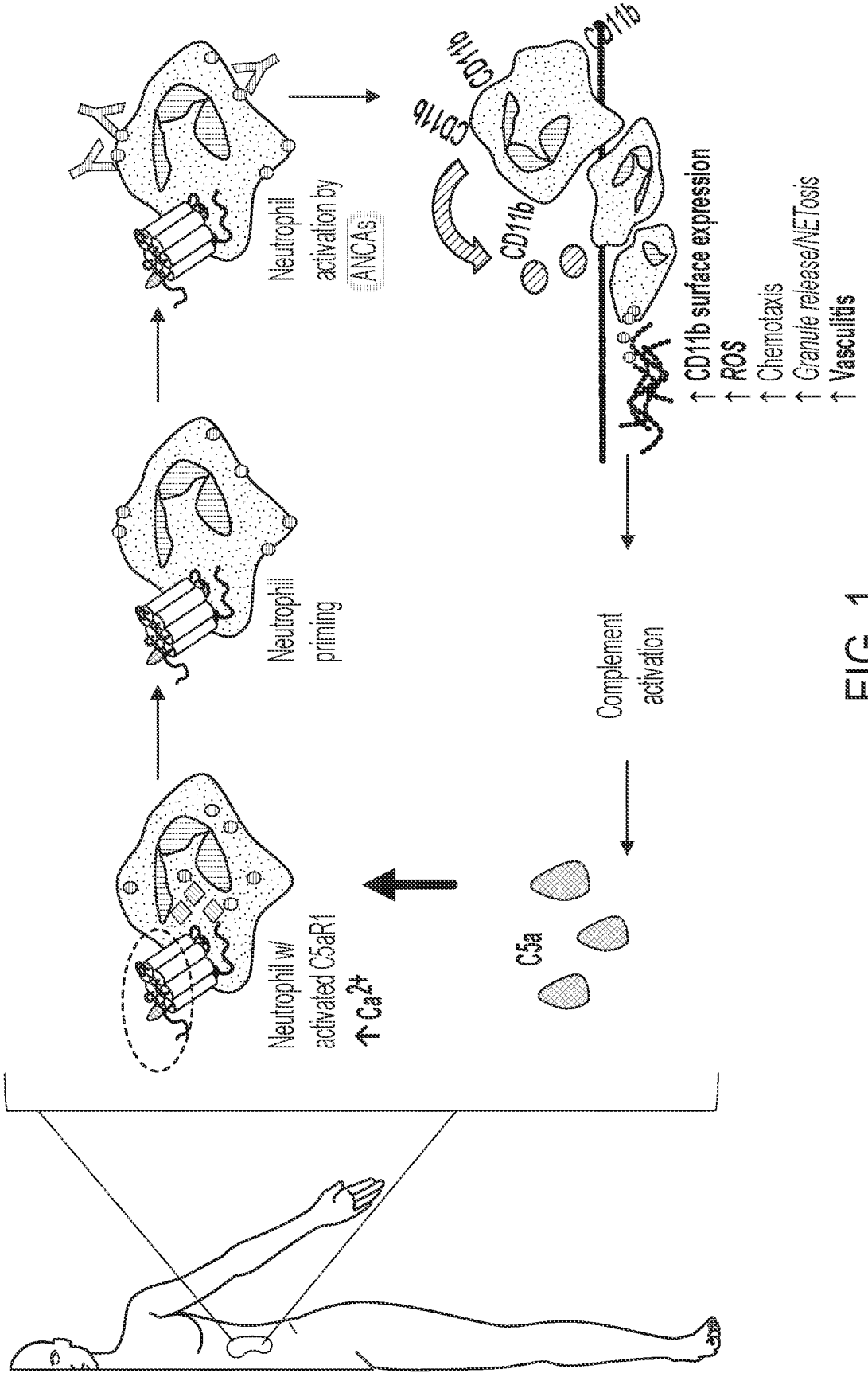

Neutrophil w/ activated C5aR1
↑ Ca^{2+}
Neutrophil priming
Neutrophil activation by ANCAs
C5a
Complement activation
CD11b
CD11b
CD11b
↑ CD11b surface expression
↑ ROS
↑ Chemotaxis
↑ Granule release/NETosis
↑ Vasculitis

FIG. 1

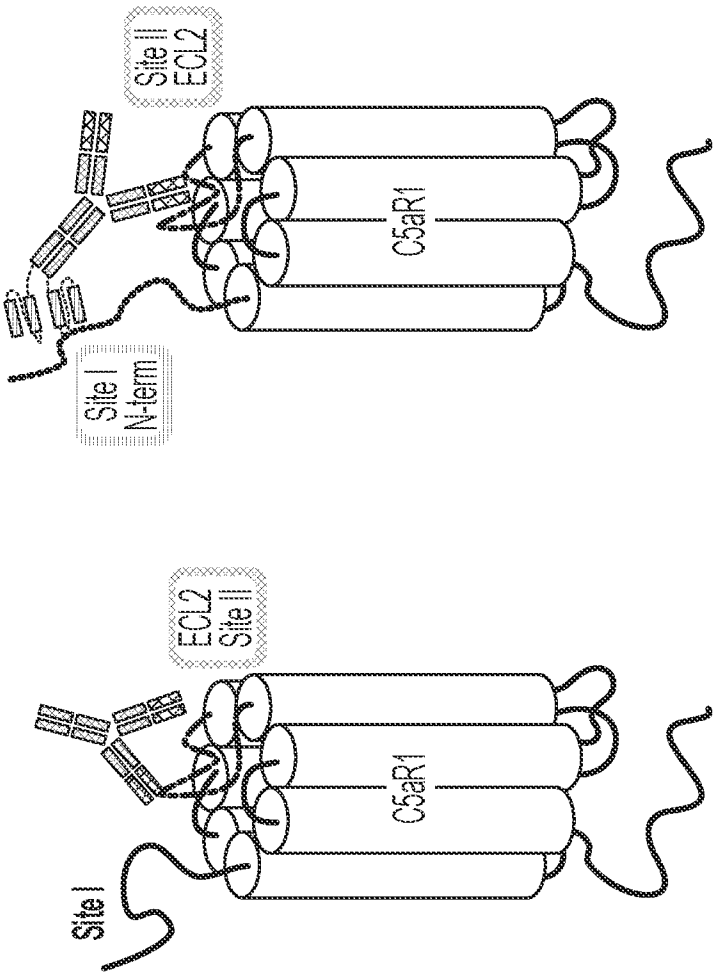


FIG. 2

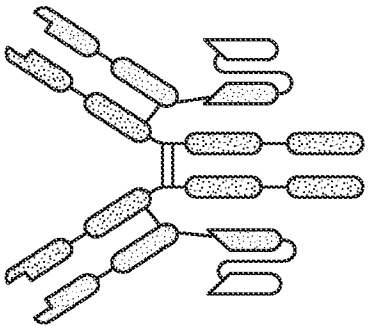


FIG. 3B

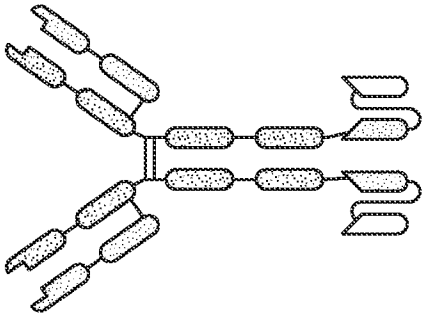


FIG. 3A

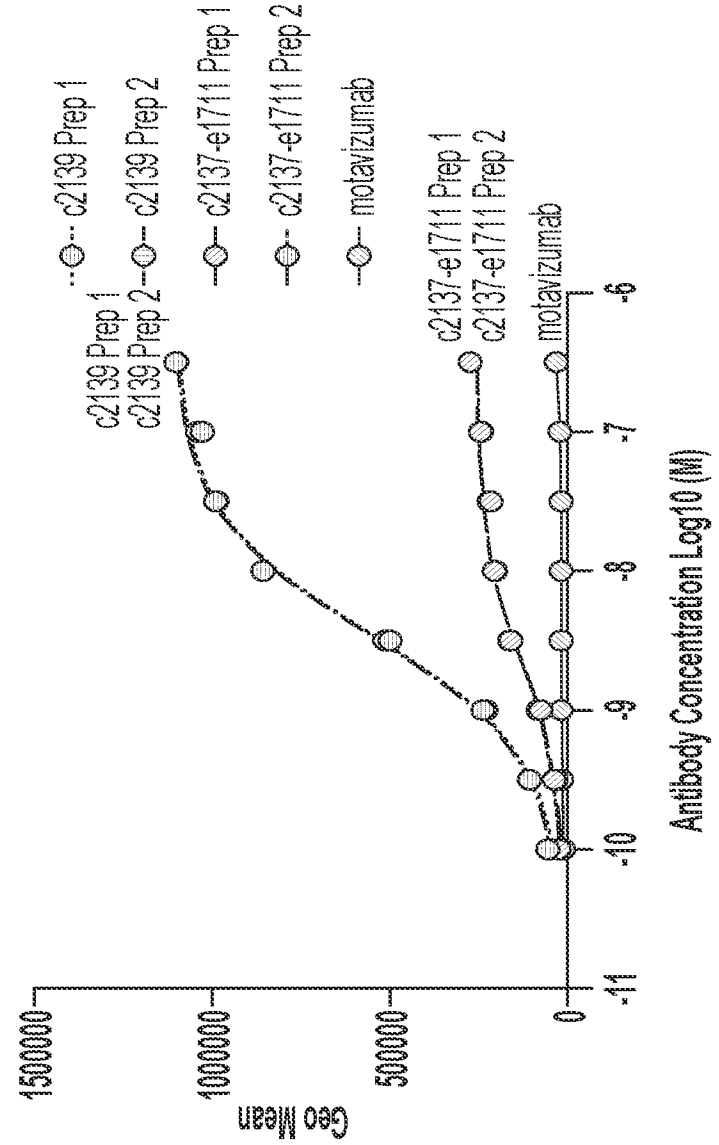

Binding Assay in U937-C5aR1 Cells
c2139 Prep 1
c2139 Prep 2
c2137-e1711 Prep 1
c2137-e1711 Prep 2
motavizumab
Geo Mean
1500000
1000000
500000
0
-11
-10
-9
-8
-7
-6
Antibody Concentration Log10 (M)

FIG. 4B

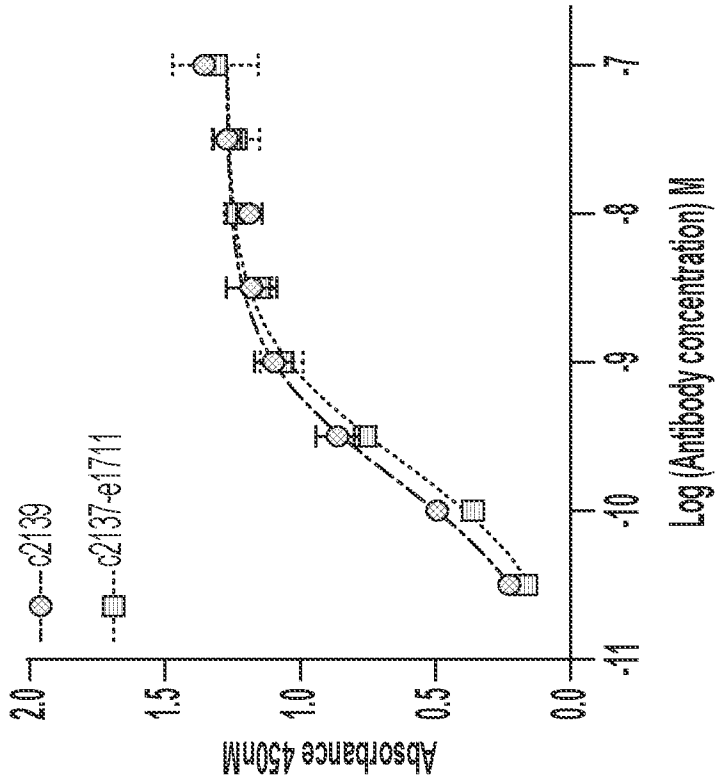

Binding by ELISA to C3aR1 VLP
c2139
c2137-e1711
Absorbance 450nM
2.0
1.5
1.0
0.5
0.0
-11
-10
-9
-8
-7
Log (Antibody concentration) M

FIG. 4A

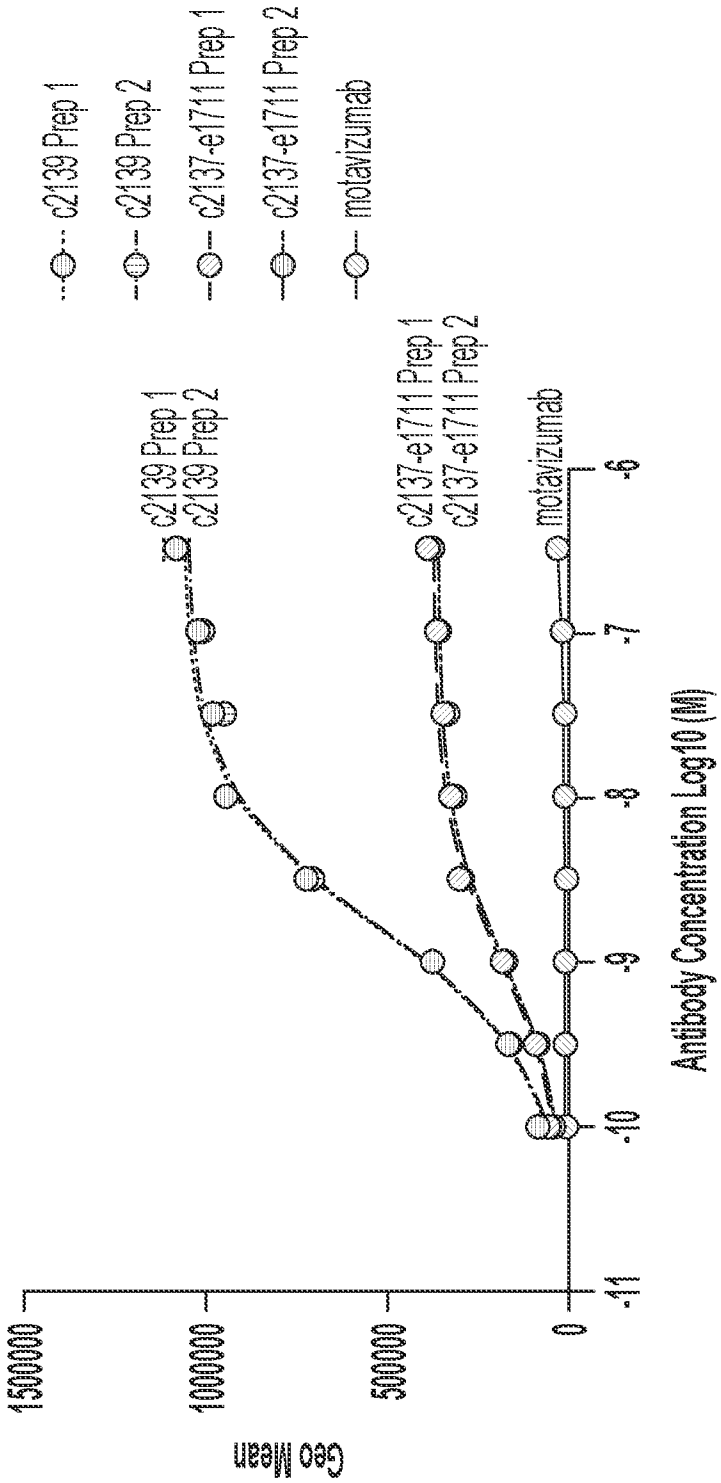

Binding Assay in Human Neutrophils
c2139 Prep 1
c2139 Prep 2
c2137-e1711 Prep 1
c2137-e1711 Prep 2
motavizumab
Geo Mean
1500000
1000000
500000
0
-11
-10
-9
-8
-7
-6
Antibody Concentration Log10 (M)

FIG. 4C

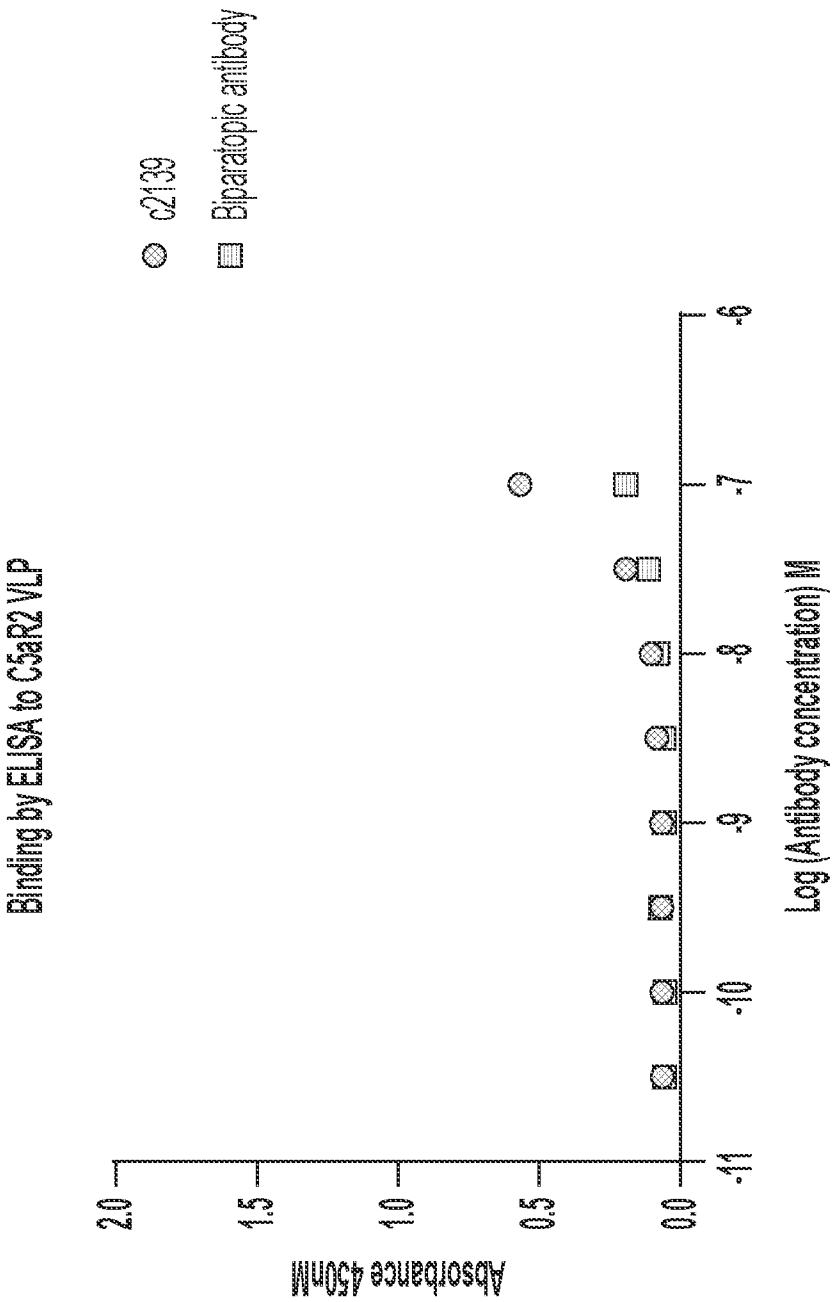

Binding by ELISA to C3aR2 VLP
c2139
Biparatopic antibody
Absorbance 450nM
2.0
1.5
1.0
0.5
0.0
-11
-10
-9
-8
-7
-6
Log (Antibody concentration) M

FIG. 5

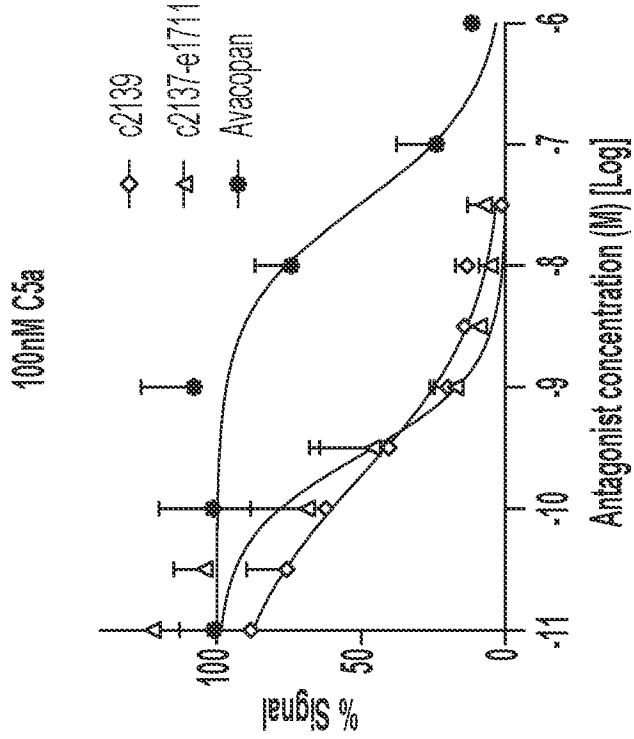

100nM C5a
c2139
c2137-e1711
Avacopan
% Signal
100
50
0
-11
-10
-9
-8
-7
-6
Antagonist concentration (M) [Log]

FIG. 6B

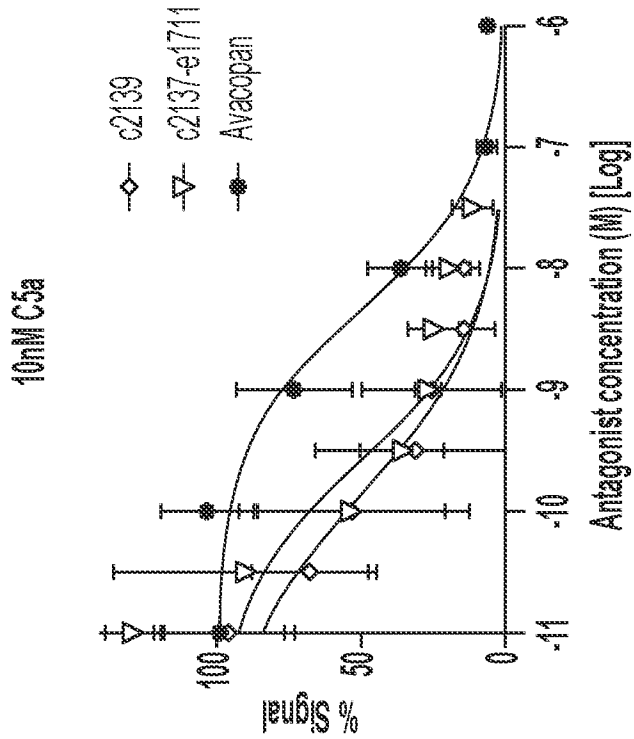

10nM C5a
c2139
c2137-e1711
Avacopan
% Signal
100
50
0
-11
-10
-9
-8
-7
-6
Antagonist concentration (M) [Log]

FIG. 6A

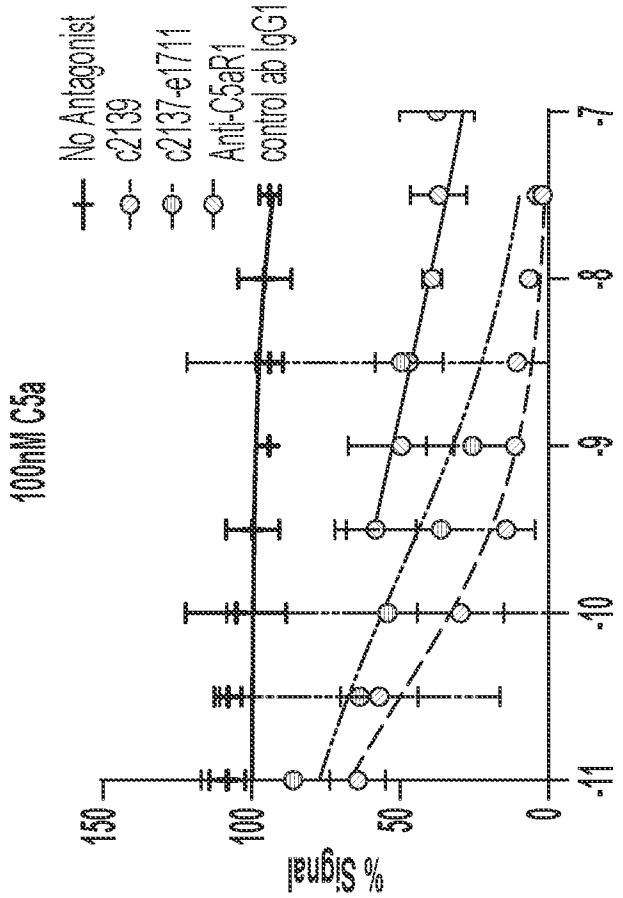

100nM C5a
No Antagonist
c2139
c2137-e1711
Anti-C5aR1
control ab IgG1
% Signal
150
100
50
0
-7
-8
-9
-10
-11
Ab Log (M)

FIG. 6D

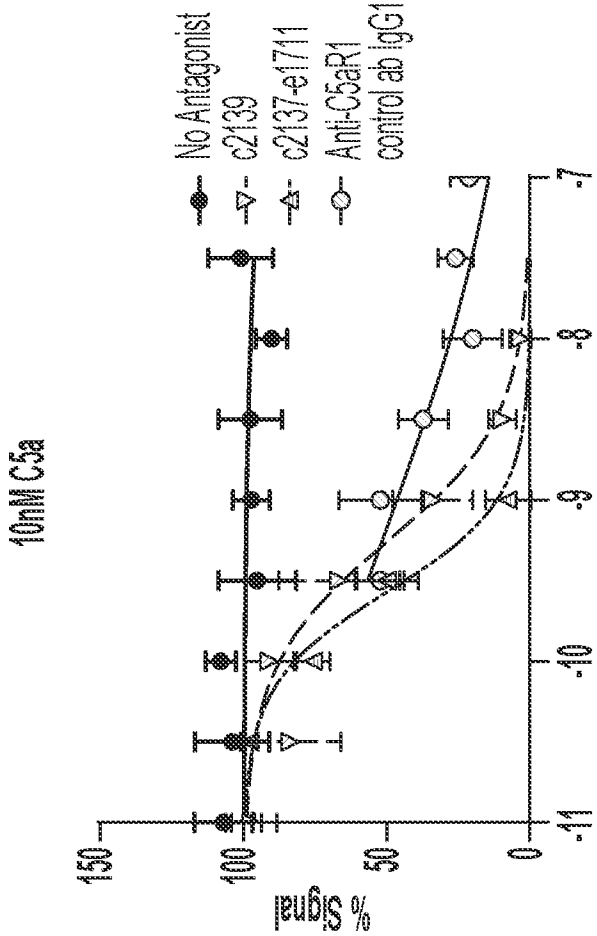

10nM C5a
No Antagonist
c2139
c2137-e1711
Anti-C5aR1
control ab IgG1
% Signal
150
100
50
0
-7
-8
-9
-10
-11
Ab Log (M)

FIG. 6C

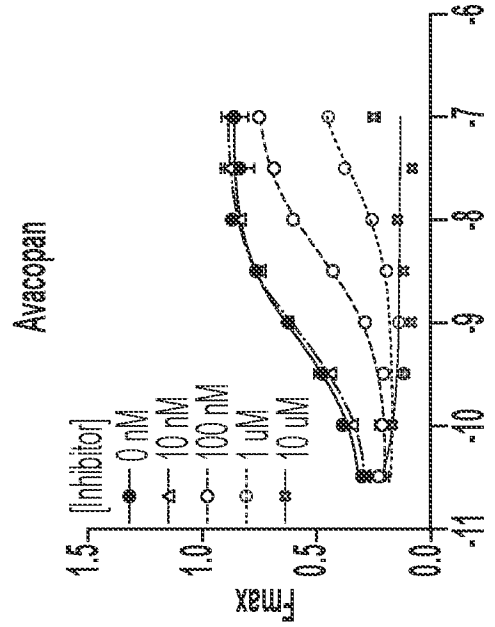


FIG. 7C

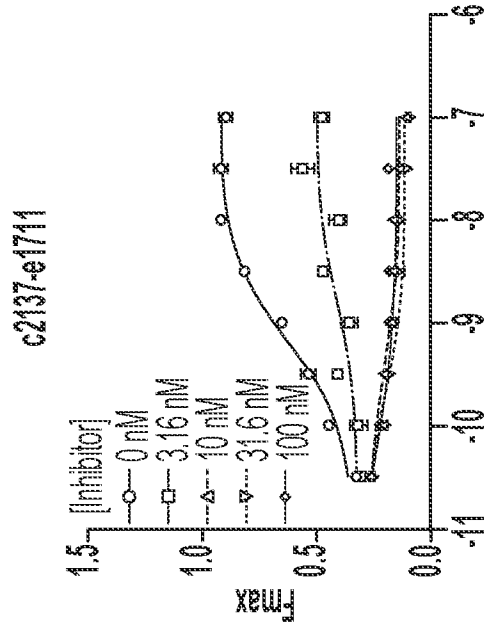


FIG. 7B

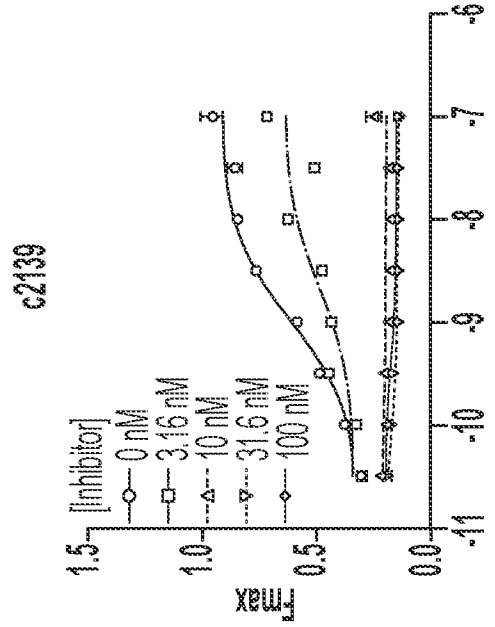


FIG. 7A

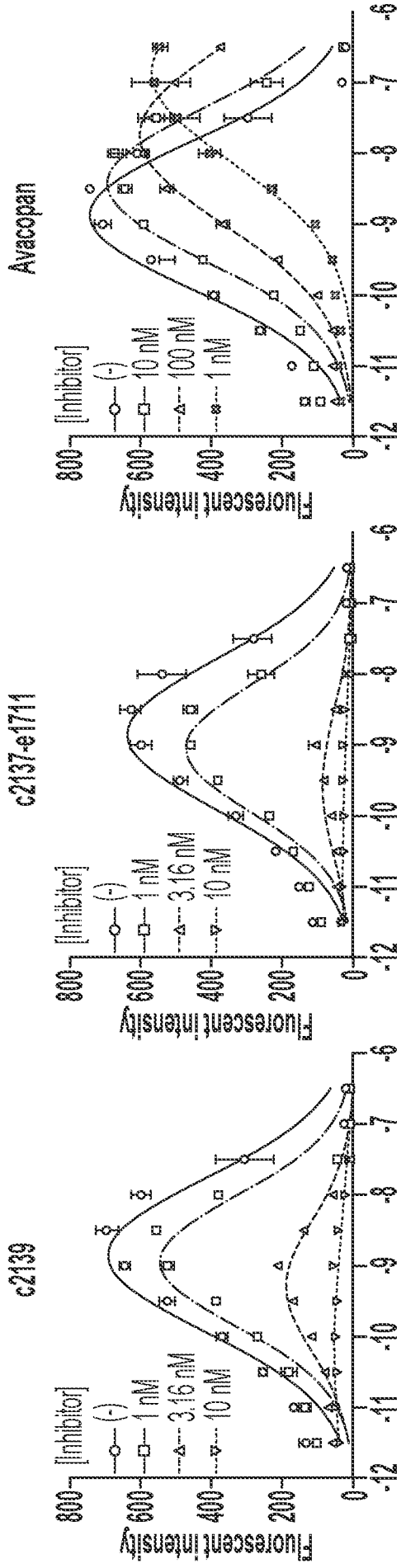


FIG. 8A

FIG. 8B

FIG. 8C

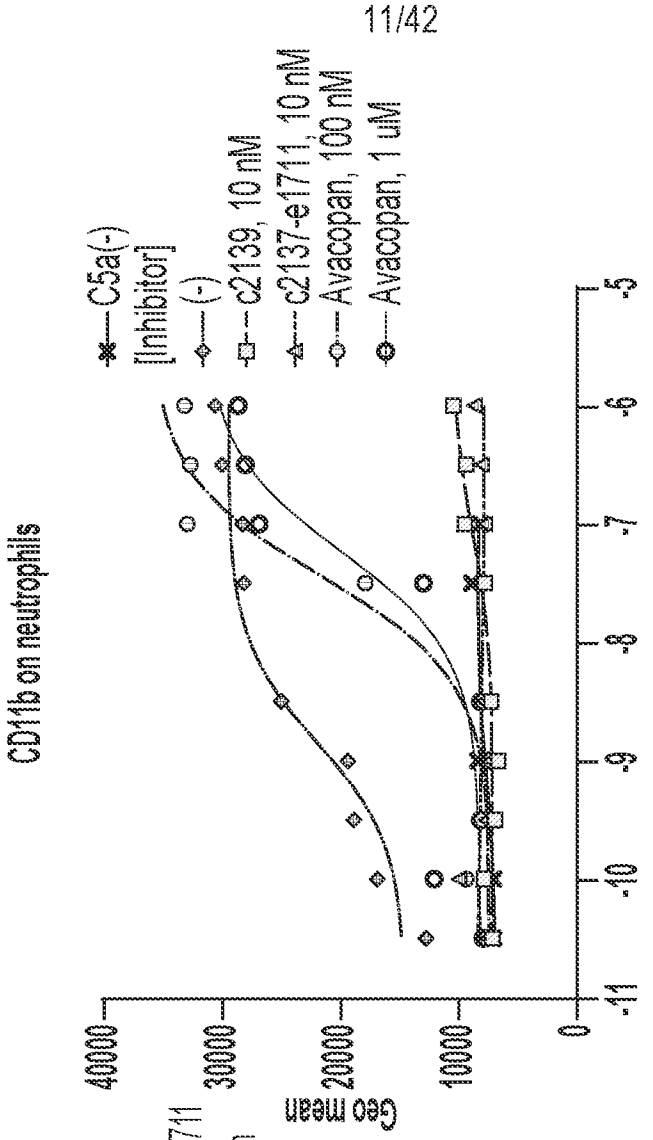

CD11b on neutrophils
C5a(-)
[Inhibitor]
(-)
c2139, 10 nM
c2137-e1711, 10 nM
Avacopan, 100 nM
Avacopan, 1 uM
Geo mean
40000
30000
20000
10000
0
-5
-6
-7
-8
-9
-10
-11
C5a M [Log]

FIG. 9B

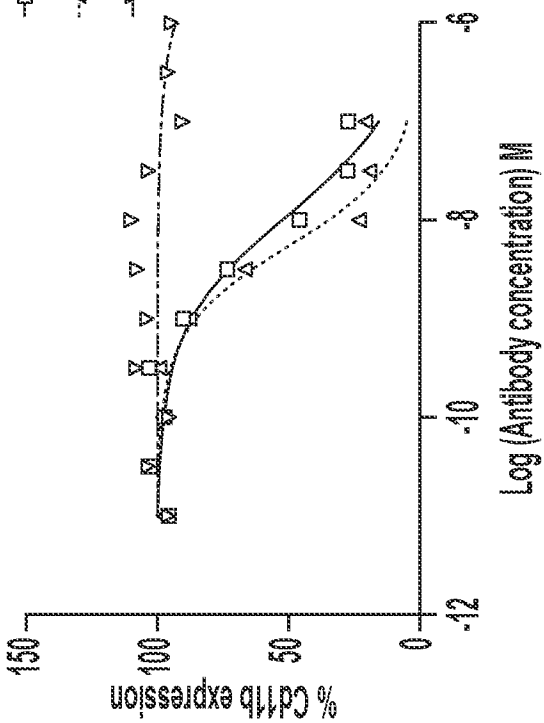

c2139
c2137-e1711
Avacopan
% Cd11b expression
150
100
50
0
-6
-8
-10
-12
Log (Antibody concentration) M

FIG. 9A

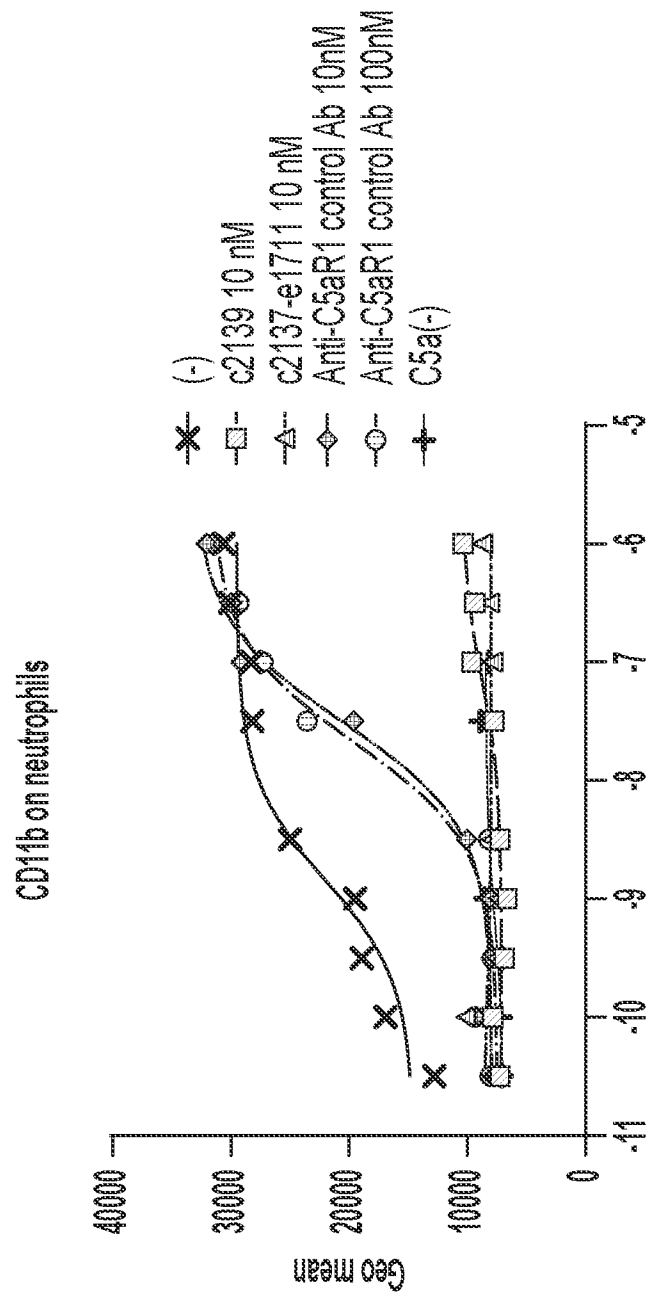

CD11b on neutrophils
Geo mean
40000
30000
20000
10000
0
-5
-6
-7
-8
-9
-10
-11
C5a III [Log]
(-)
c2139 10 nM
c2137-e1711 10 nM
Anti-C5aR1 control Ab 10nM
Anti-C5aR1 control Ab 100nM
C5a(-)

FIG. 9C

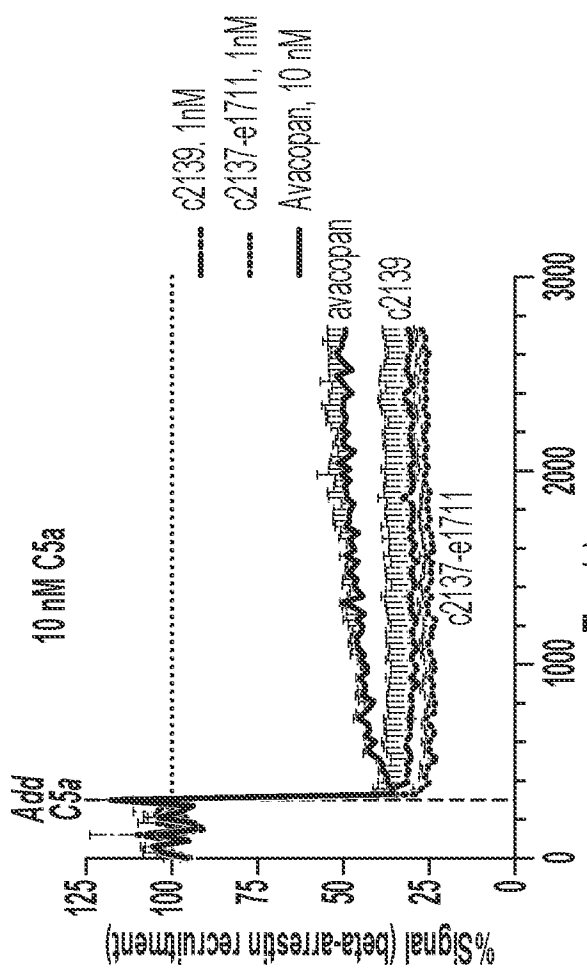


FIG. 10A

FIG. 10B

FIG. 10C

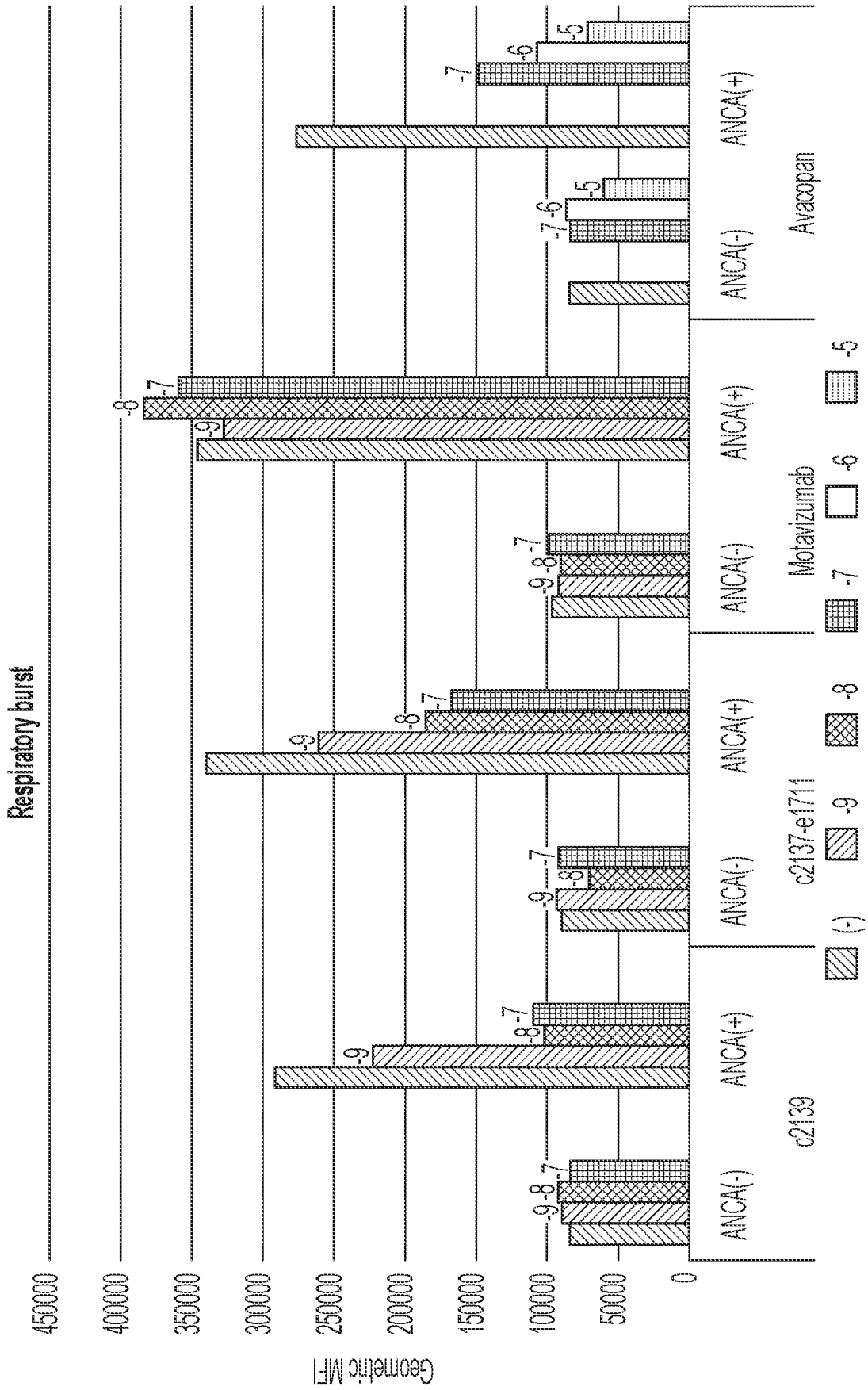

Respiratory burst
Geometric MFI
450000
400000
350000
300000
250000
200000
150000
100000
50000
0
c2139
c2137-e1711
Motavizumab
Avacopan
ANCA(-)
ANCA(+)
(-)
-9
-8
-7
-6
-5

FIG. 11

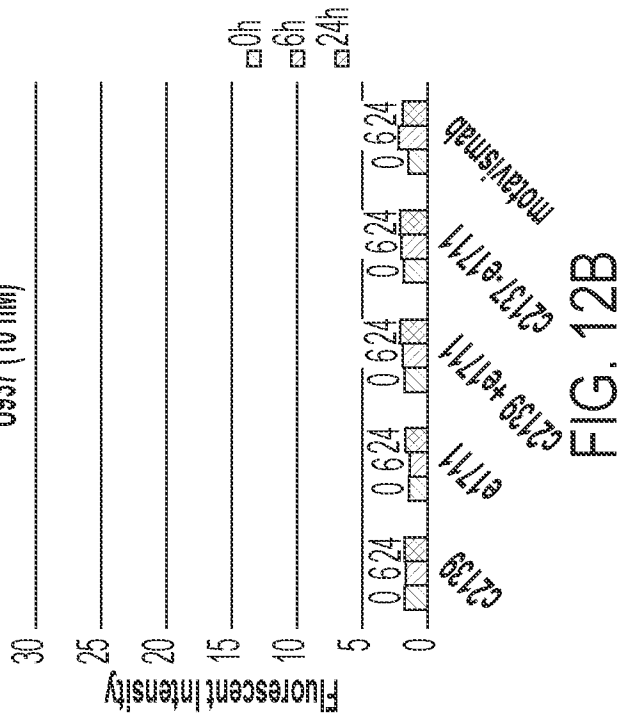


FIG. 12A

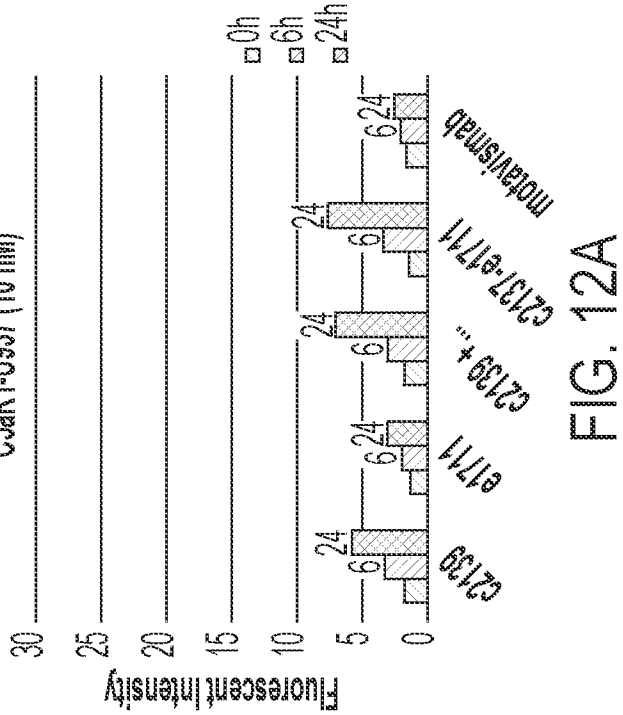


FIG. 12B

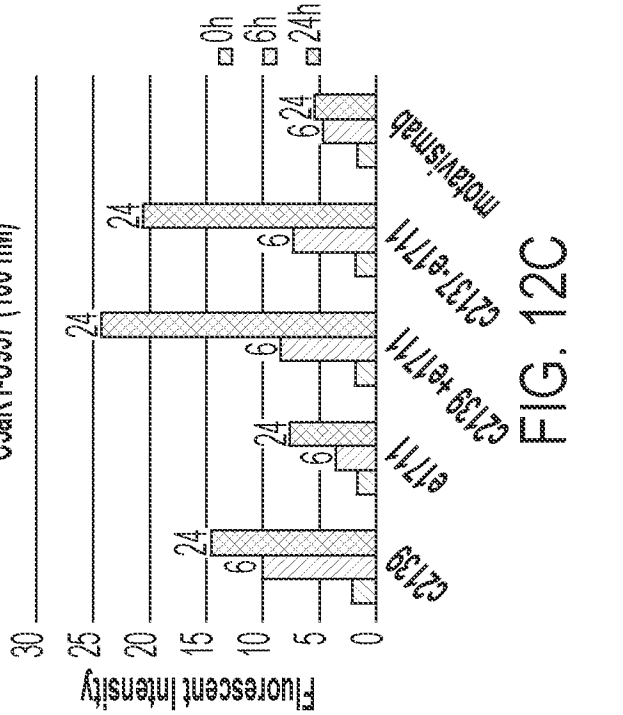


FIG. 12C

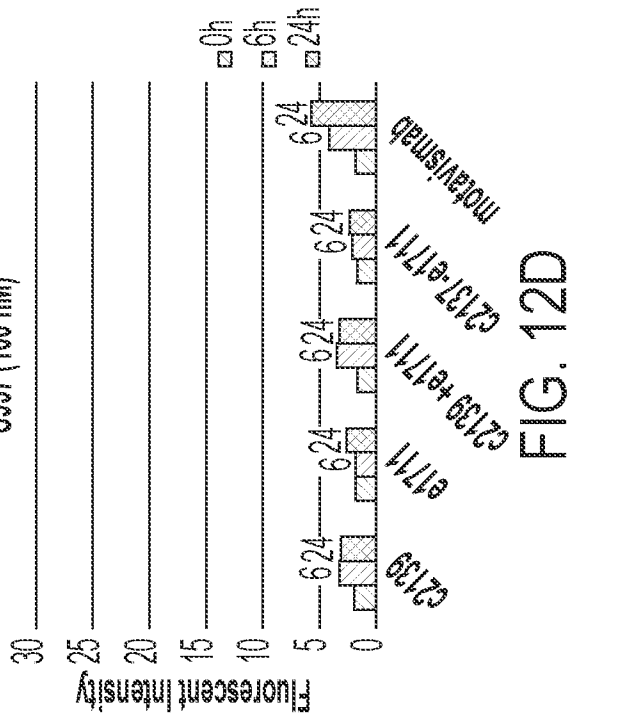


FIG. 12D

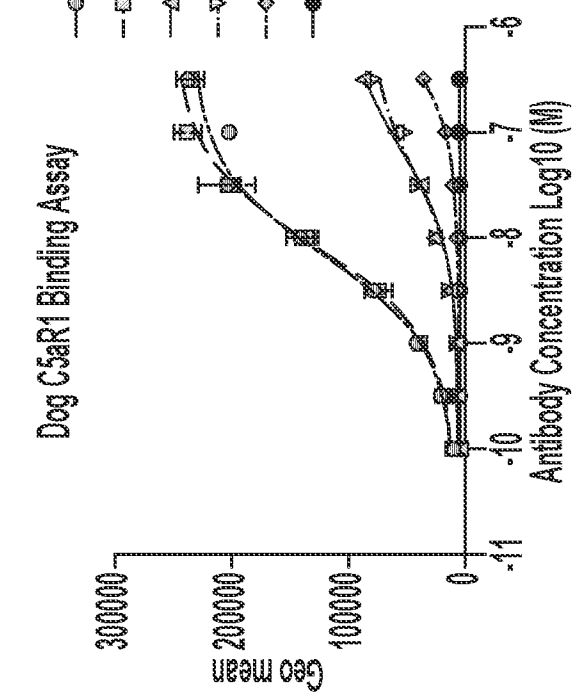


FIG. 13B

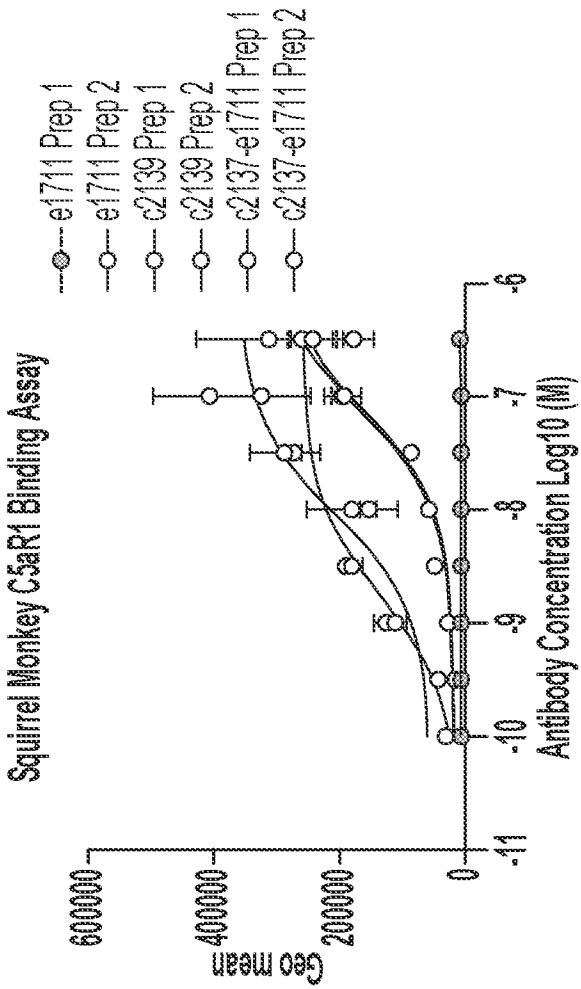
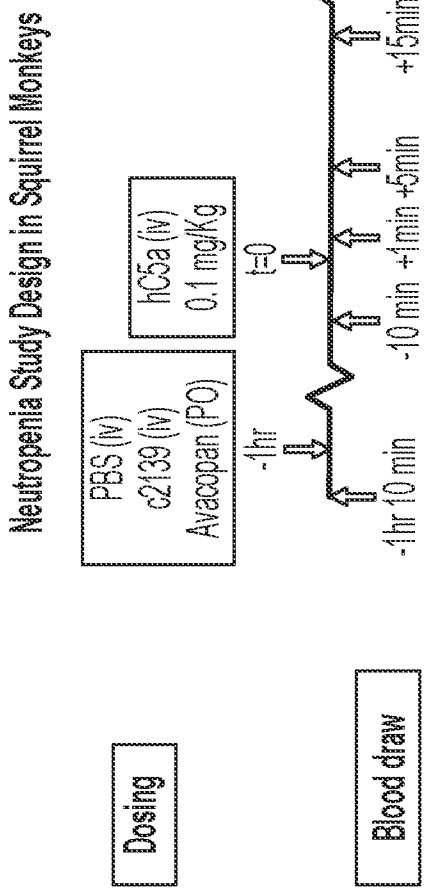


FIG. 13A



Group	N	Dose Route	Test Article	Dose (mg/kg)
1	8	IV	Vehicle (PBS)	0
2	8	IV	c2139 (PBS)	10
3	8	PO	Avacopan (Solutol/PEG400 30:70)	30
Endpoints	Body Weights and clinical observations before and after experiment Neutrophil Counts: Pre-TA, Pre-C5a Dose, Post-C5a Dose up to 6 hr			

FIG. 14A

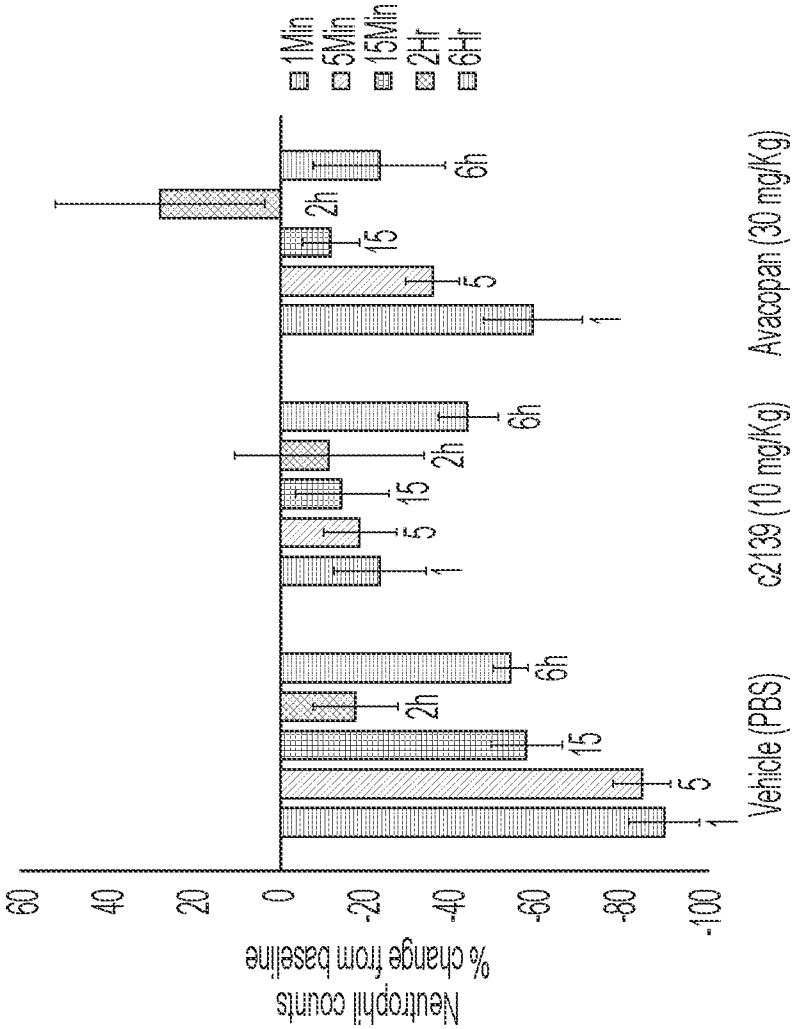


FIG. 14C

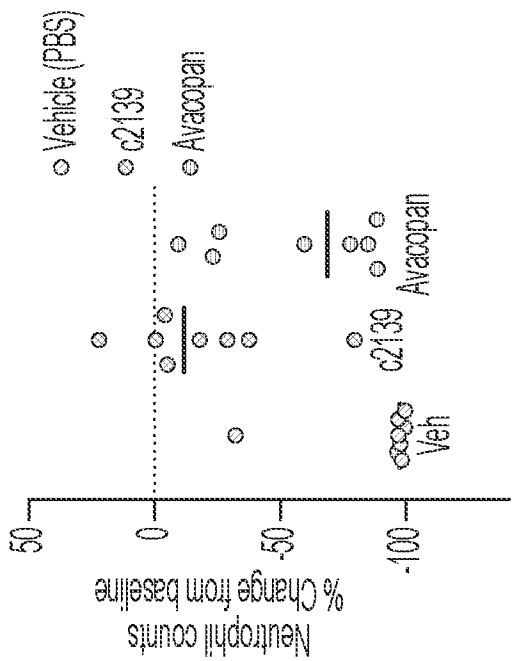
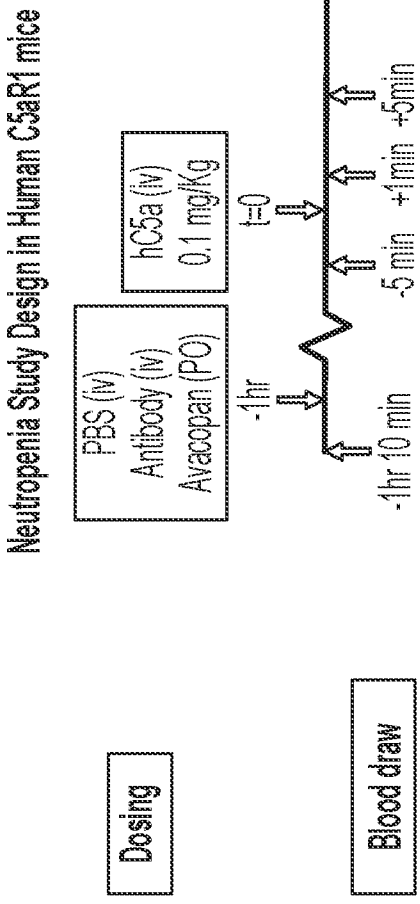


FIG. 14B



Group	N	Dose Route	Test Article	Dose (mg/kg)
1	5	IV	Vehicle (PBS)	0
2	5	IV	c2139 (PBS)	20
3	5	IV	c2137-e1711(PBS)	20
4	5	IV	Motavizumab (PBS)	20
5	5	PO	Avacopan(Solutol/PEG400 30:70)	30
Endpoints	Blood collection: Pre-TA, Pre-C5a Dose, Post-C5a Dose up to 2 hr FACS analysis for Markers 7AAD, CD45, CD11b, Ly6G+, mCD88, hCD88			

FIG. 15A

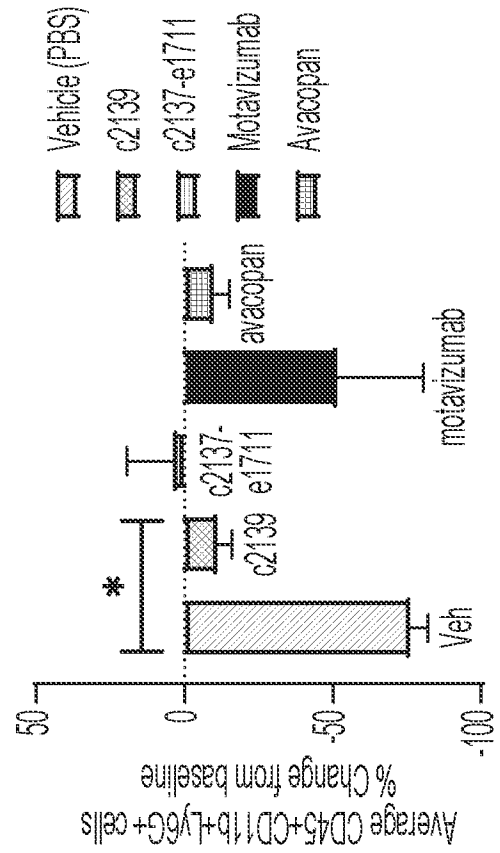


FIG. 15C

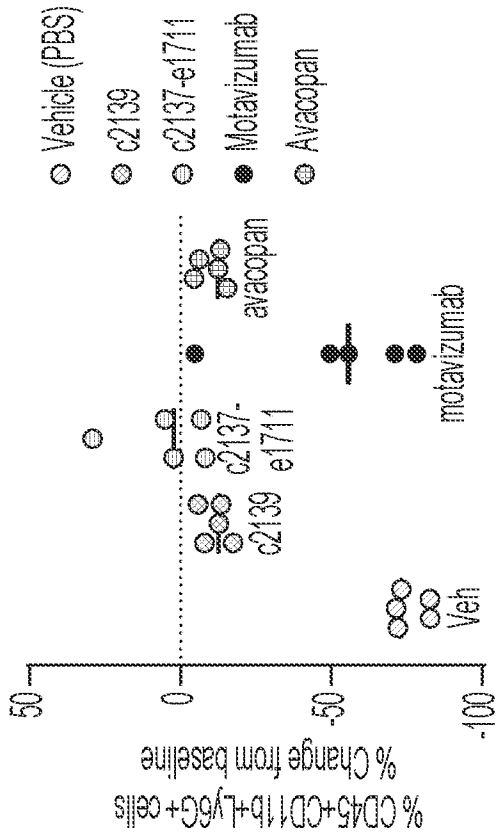


FIG. 15B

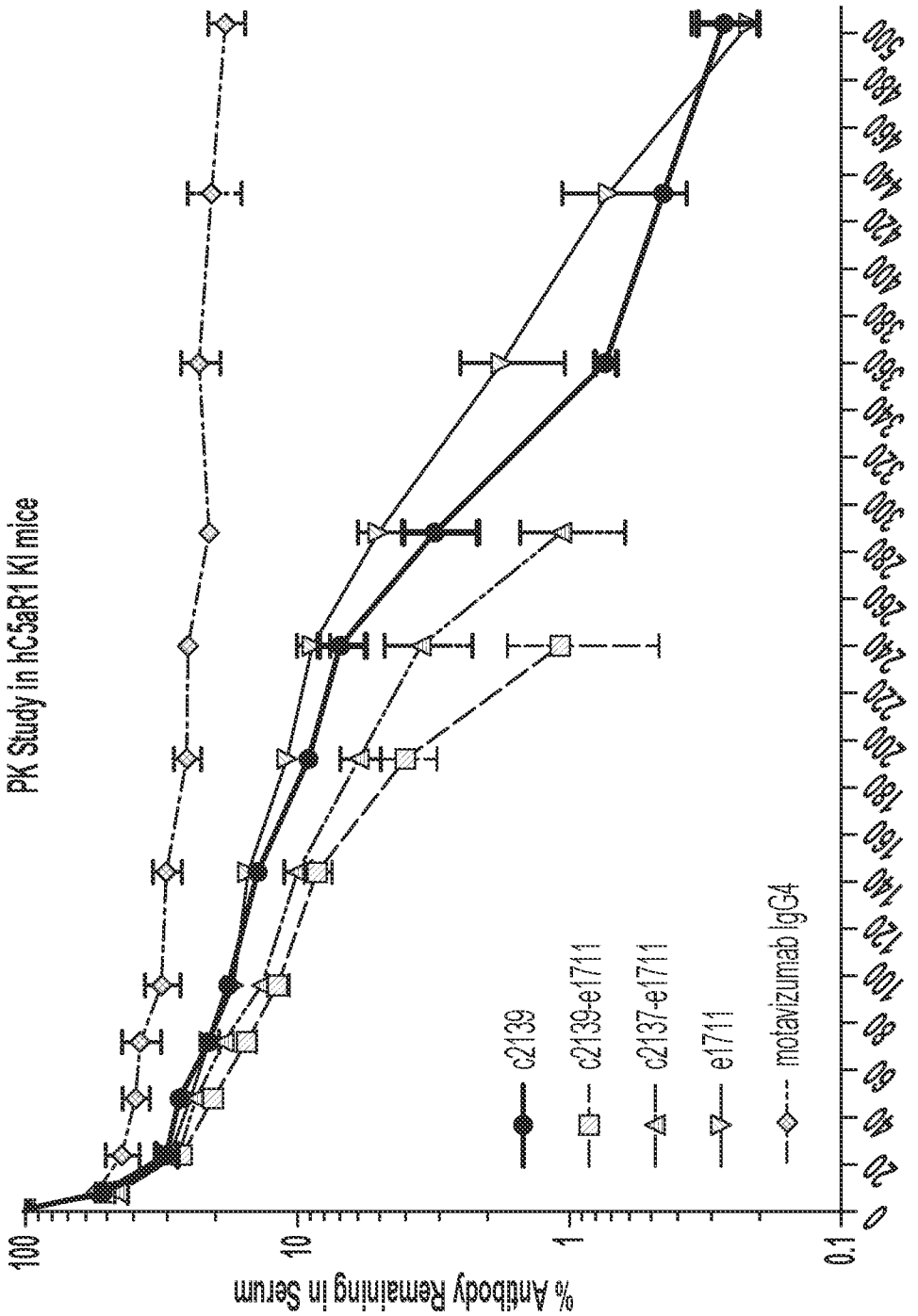

PK Study in hC5aR1 KI mice
% Antibody Remaining in Serum
100
10
1
0.1
Hours
0
20
40
60
80
100
120
140
160
180
200
220
240
260
280
300
320
340
360
380
400
420
440
460
480
500
c2139
c2139-e1711
c2137-e1711
e1711
motavizumab IgG4

FIG. 16A

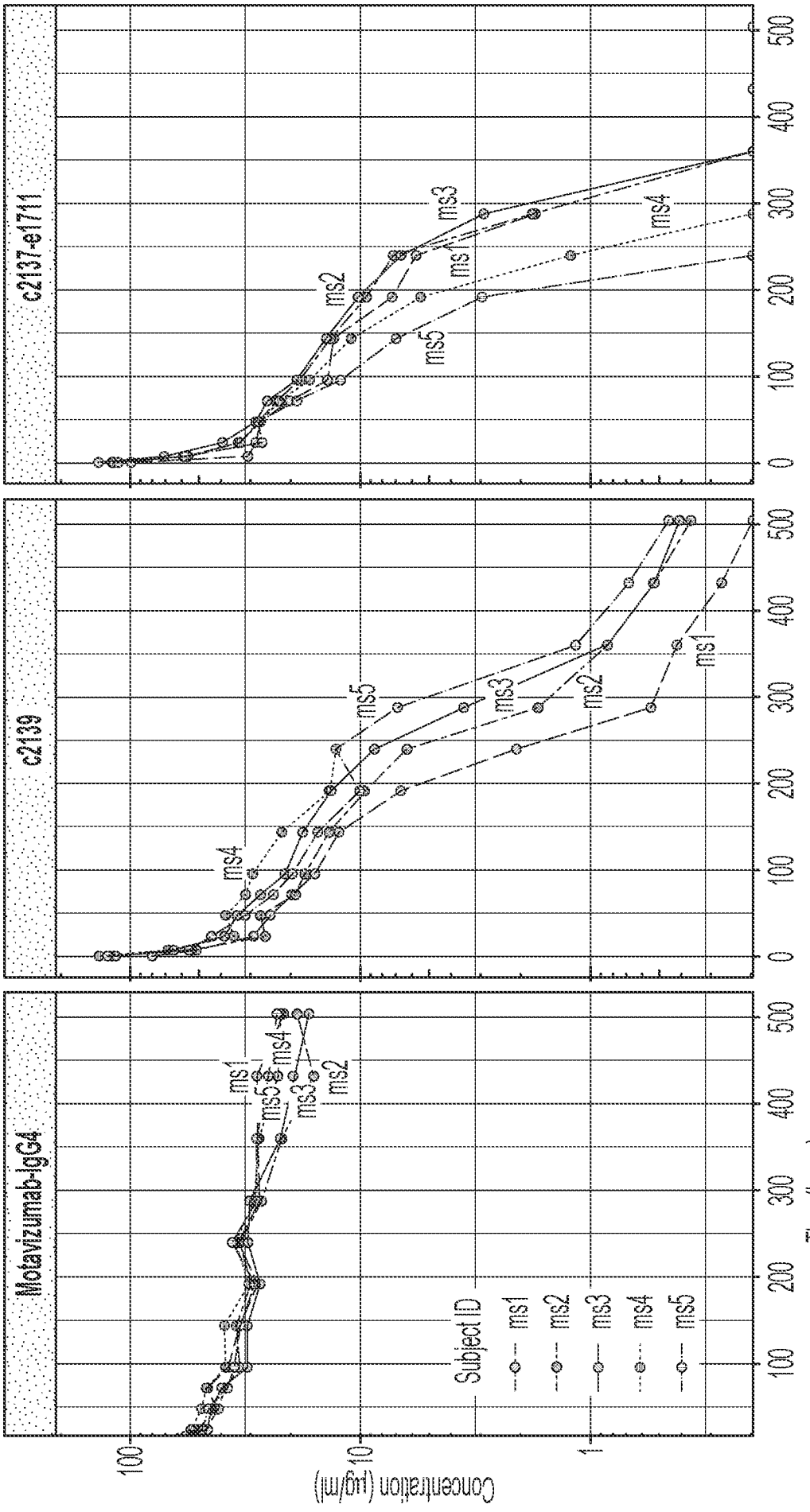


FIG. 16B

FIG. 16C

FIG. 16D

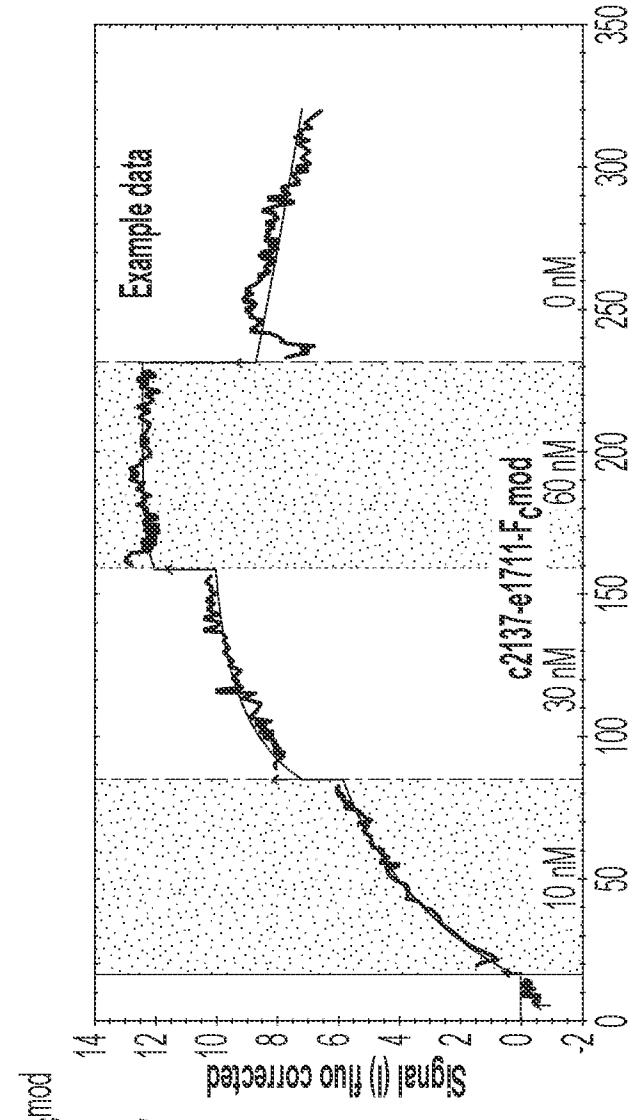
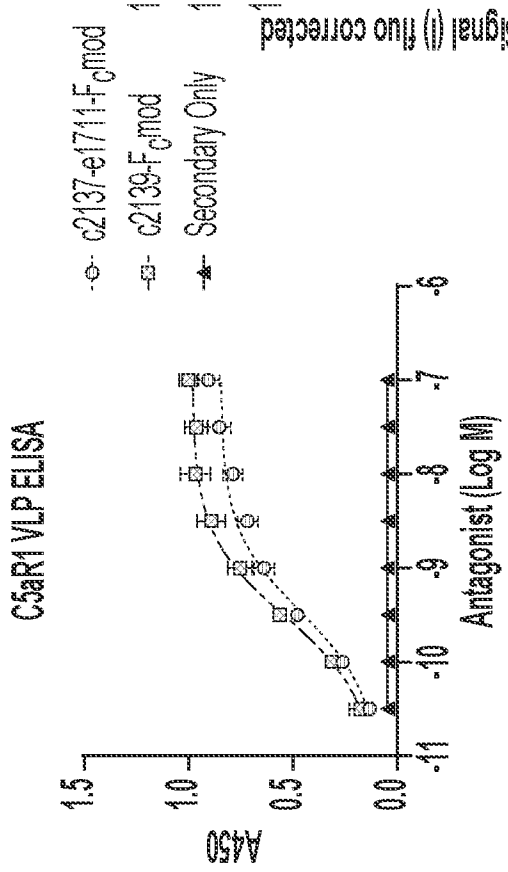


FIG. 17B



Antibody	EC_{50}(nM)
C2139-$F_c$mod	0.31
C2137-e1711-Fcmod	0.34

FIG. 17A

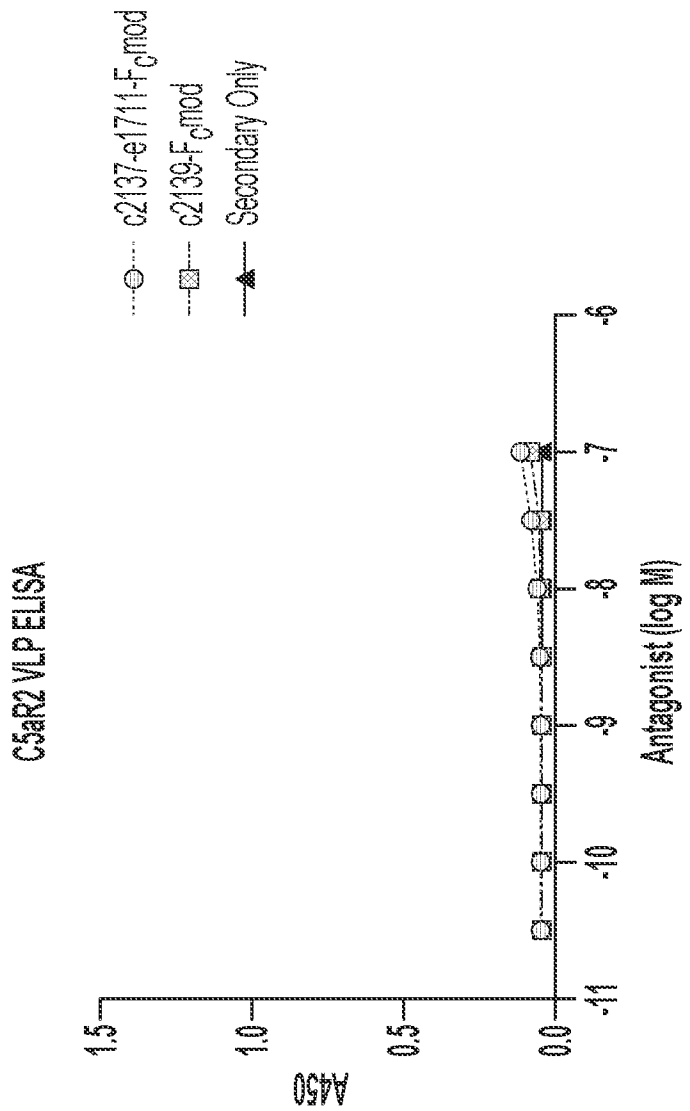
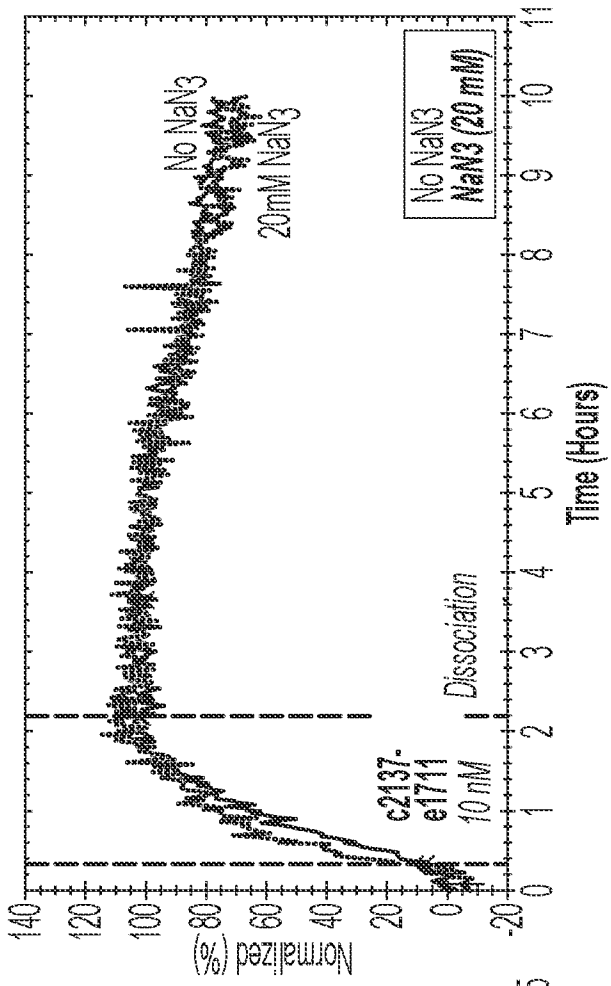
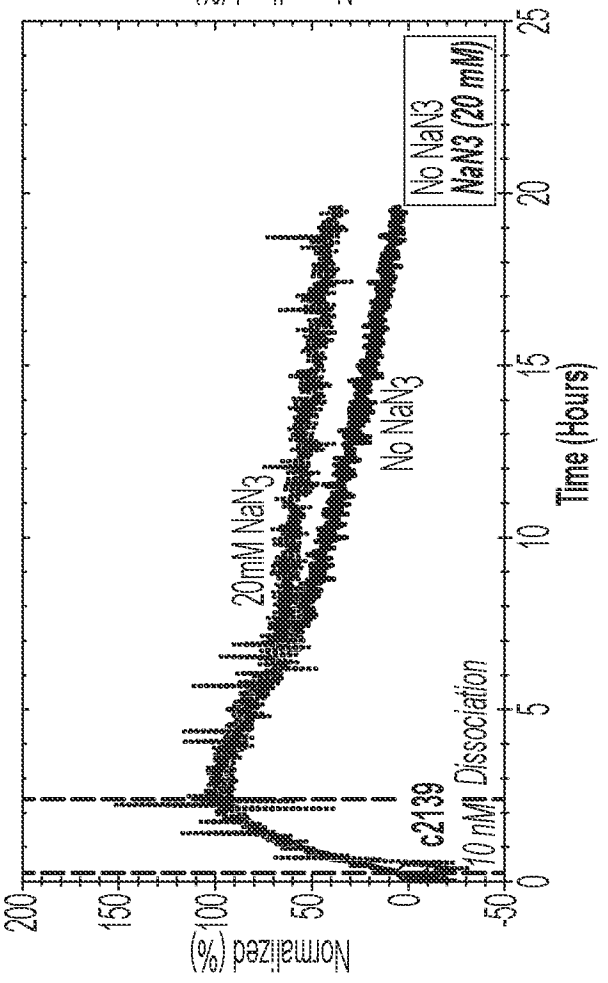

C5aR2 VLP ELISA
c2137-e1711-Fcmod
c2139-Fcmod
Secondary Only
A450
1.5
1.0
0.5
0.0
-6
-7
-8
-9
-10
-11
Antagonist (log M)

FIG. 17C



	Without NaN3	With NaN3
Ka (1/(M*s))	4.38e3	2.15e4
Kd (1/s)	1.36e-5	2.20e-5
KD (nM)	0.423	0.539

FIG. 18B



	Without NaN3	With NaN3
Ka (1/(M*s))	2.68e4	2.82e4
Kd (1/s)	3.4e-5	1.43e-5
KD (nM)	1.27	0.507

FIG. 18A

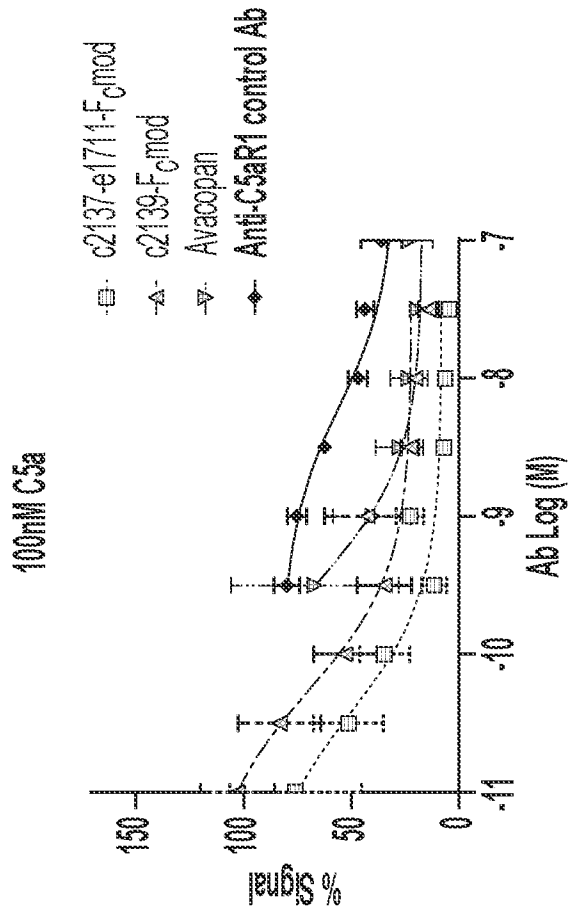


FIG. 19B

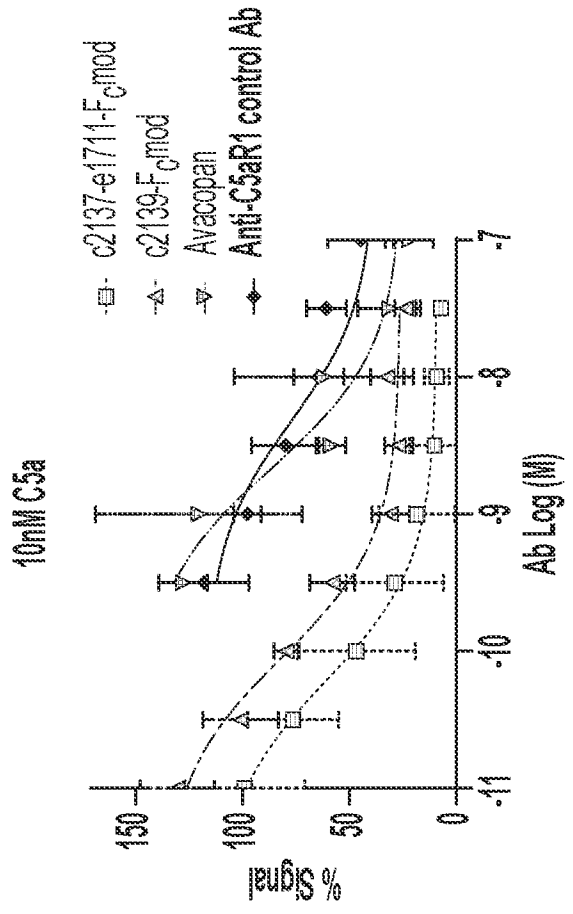


FIG. 19A

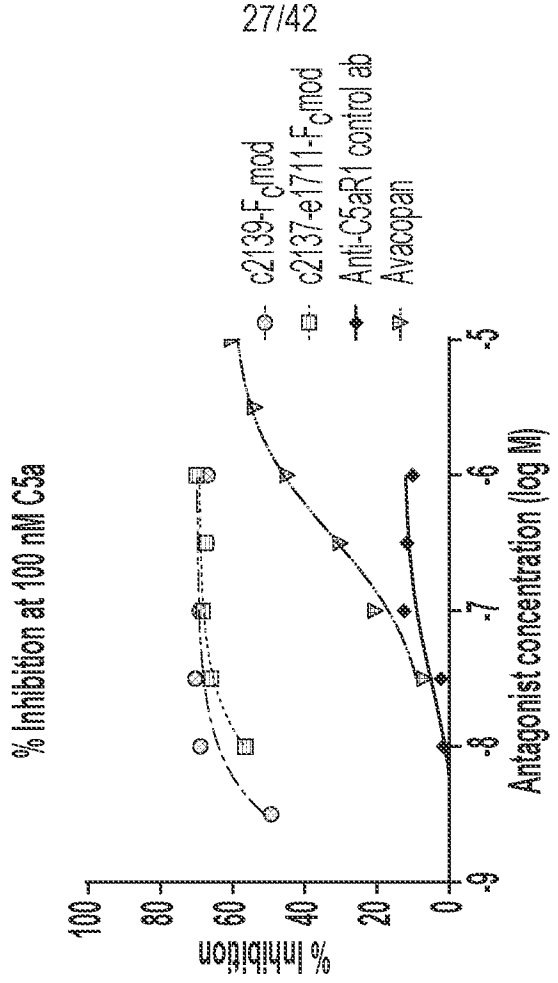


FIG. 20B

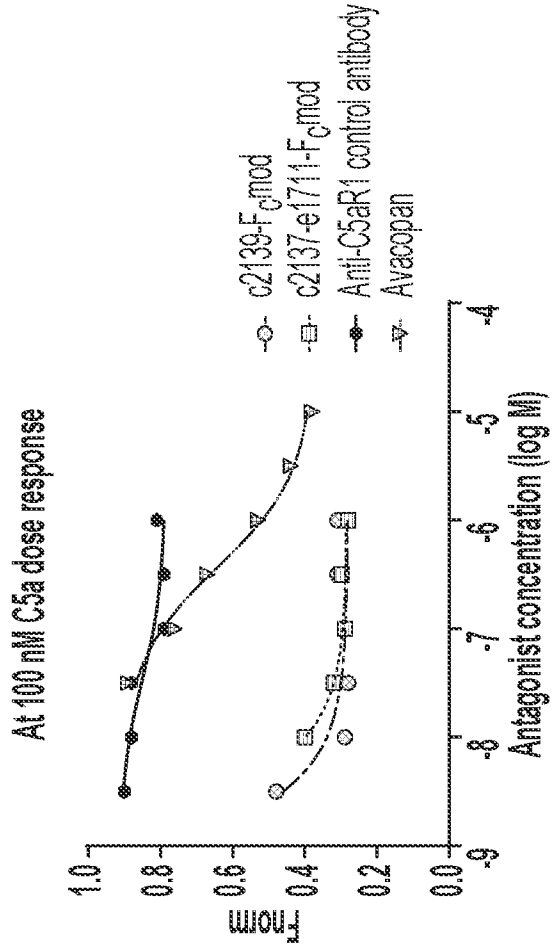


FIG. 20A

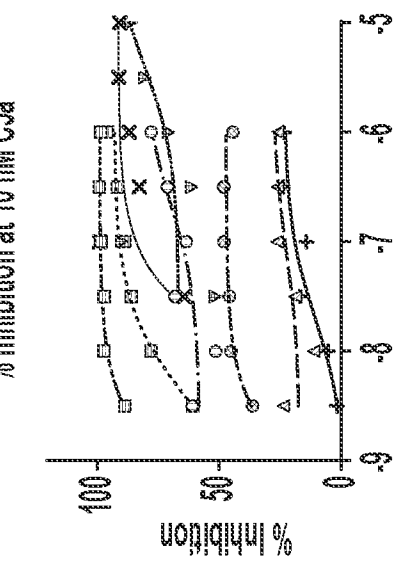


FIG. 21A

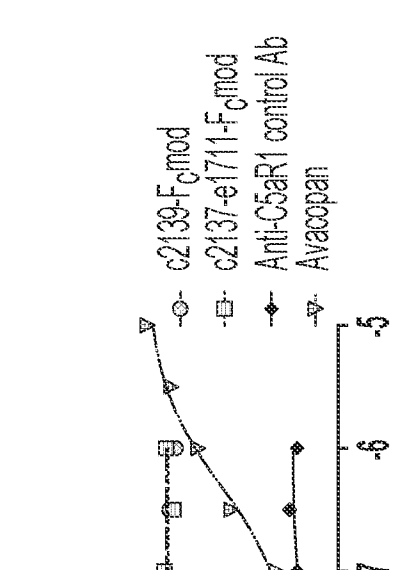


FIG. 21B

FIG. 21C

FIG. 21D

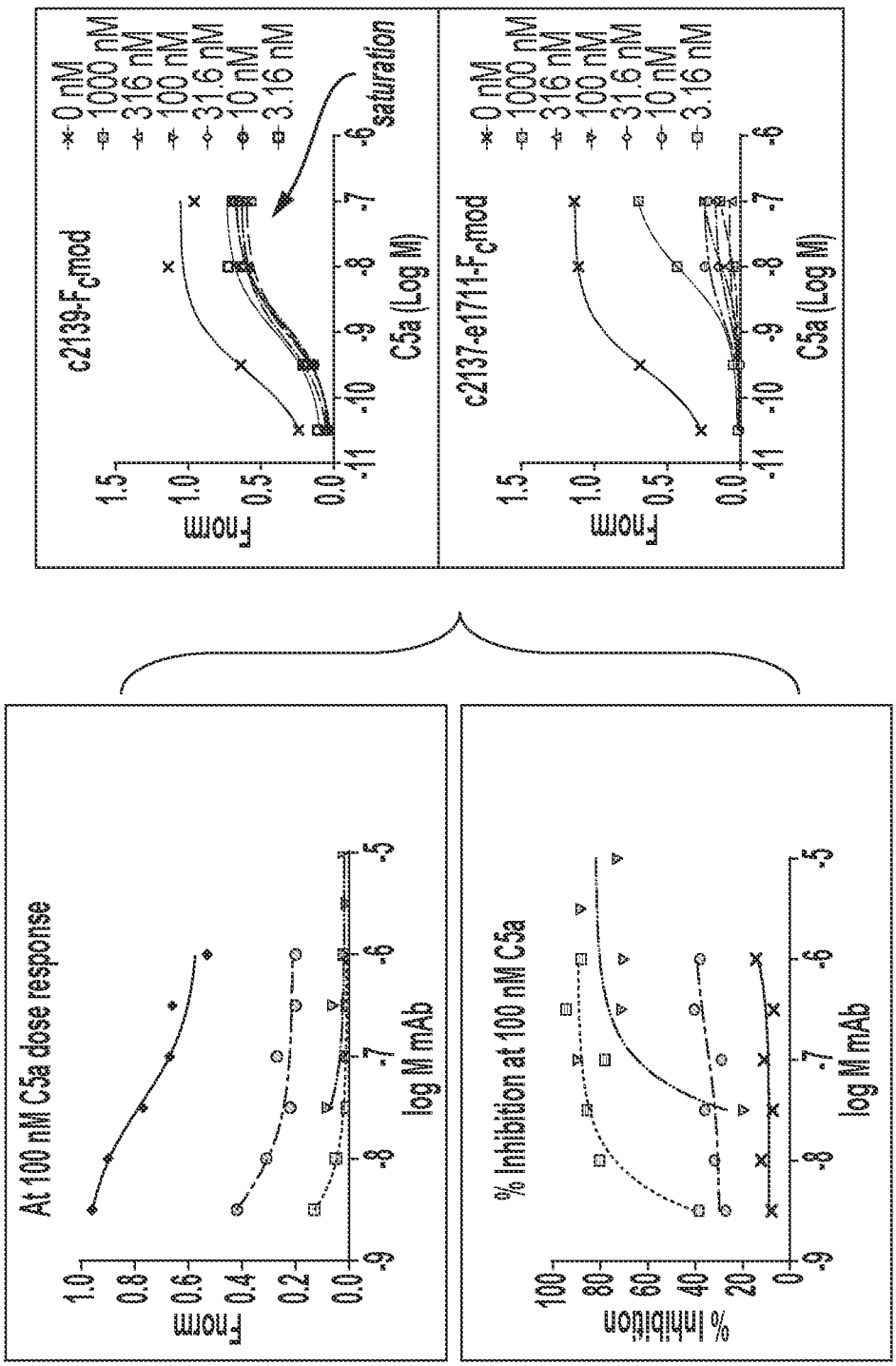

c2139-Fcmod
c2137-e1711-Fcmod
0 nM
1000 nM
316 nM
100 nM
31.6 nM
10 nM
3.16 nM
saturation
fraction
C5a (Log M)
At 100 nM C5a dose response
% Inhibition at 100 nM C5a
% Inhibition
log M mAb
c2139-Fcmod, 3hr
c2137-e1711-Fcmod, 3hr
Anti-C5aR1 control Ab, 3hr
Avacopan, 3hr

FIG. 22A

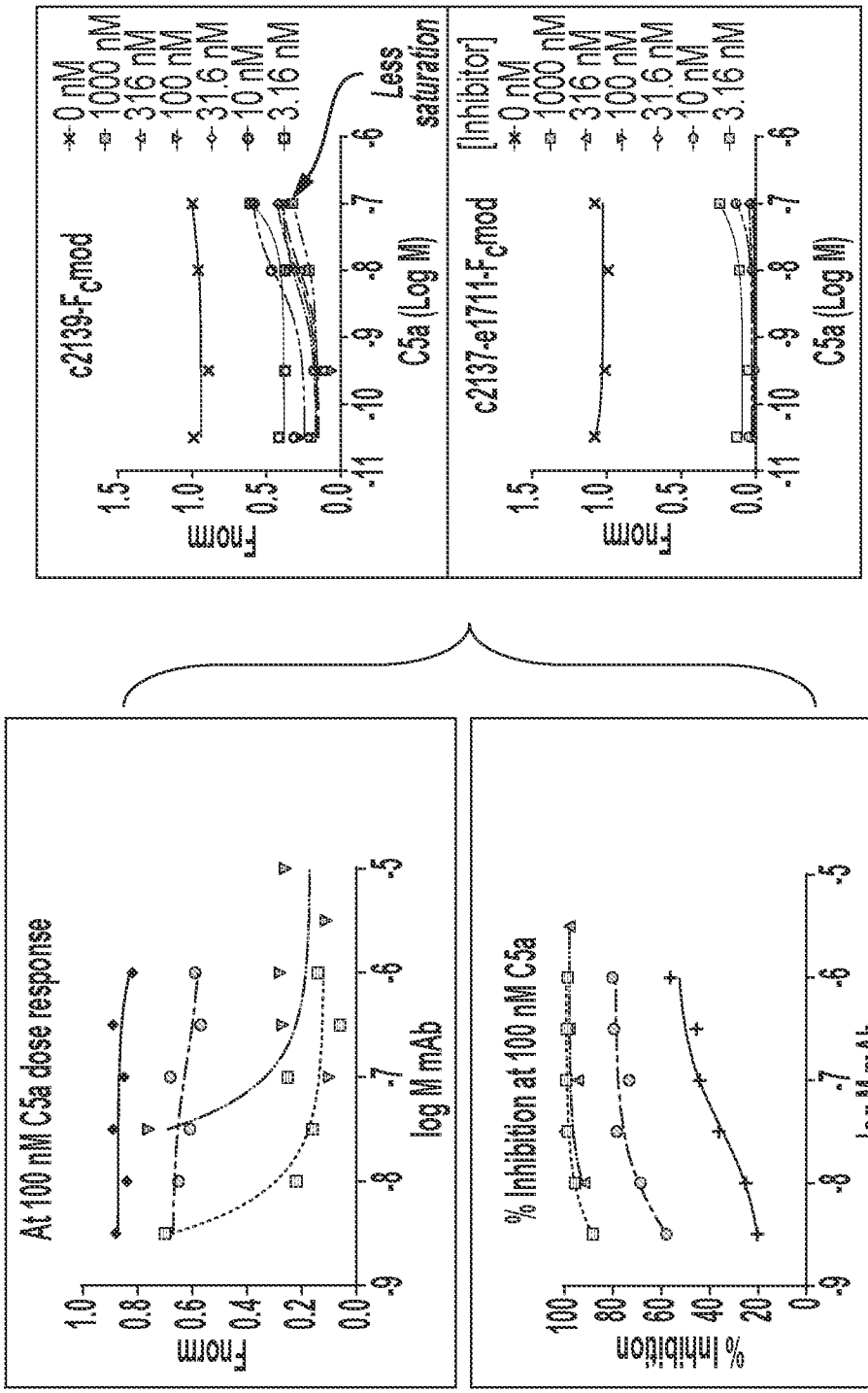

c2139-Fcmod
0 nM
1000 nM
316 nM
100 nM
31.6 nM
10 nM
3.16 nM
Less saturation
C5a (Log M)
fraction
c2137-e1711-Fcmod
[Inhibitor]
At 100 nM C5a dose response
log M mAb
% Inhibition at 100 nM C5a
% Inhibition
c2139-Fcmod, 3hr
c2137-e1711-Fcmod, 3hr
Anti-C5aR1 control Ab, 3hr
Avacopan, 3hr

FIG. 22B

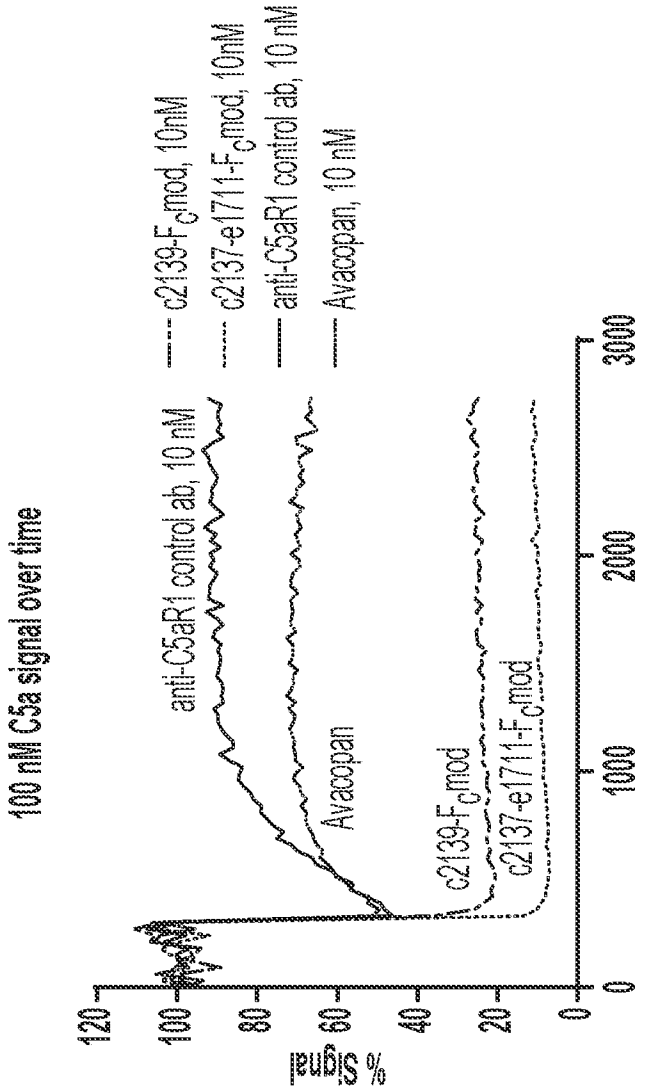


FIG. 23B

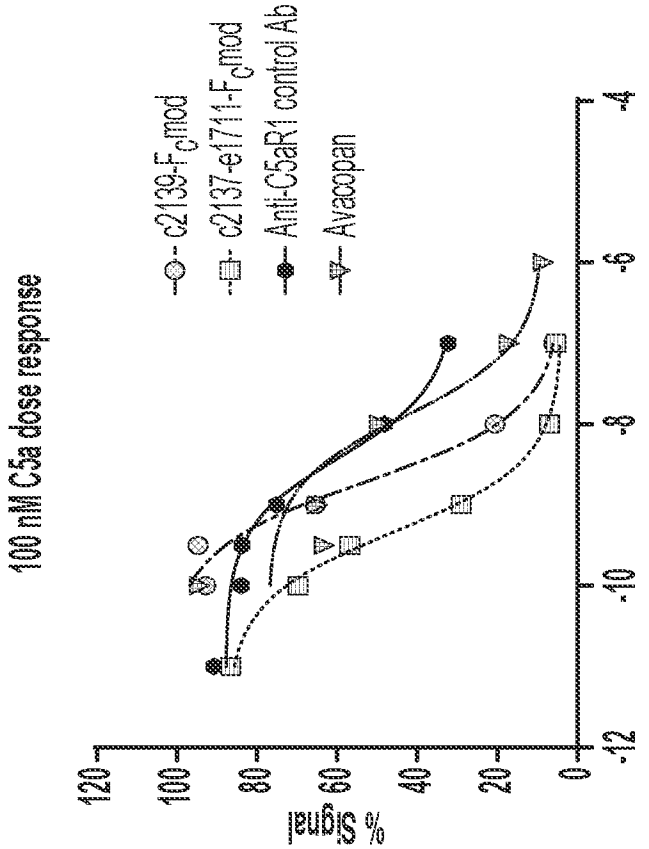


FIG. 23A

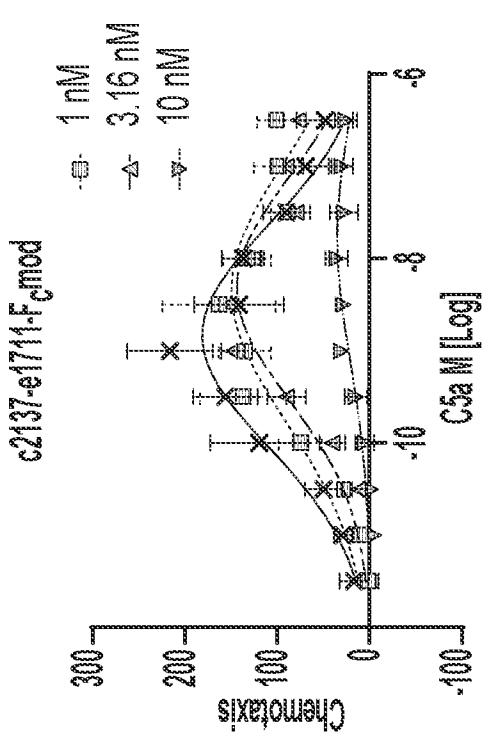


FIG. 24B

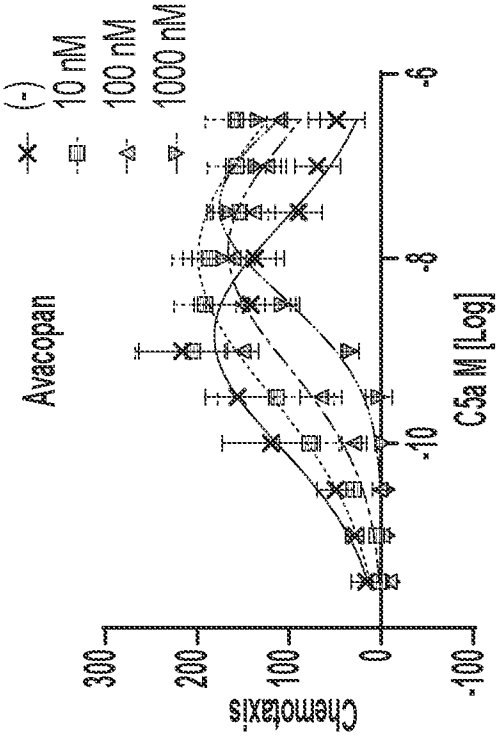


FIG. 24D

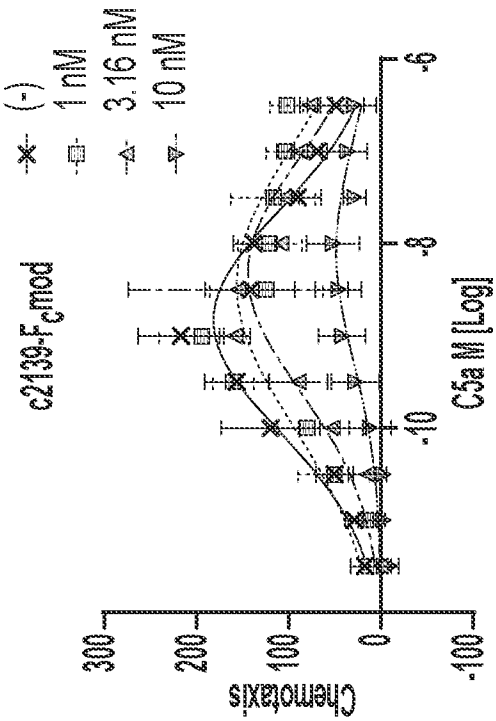


FIG. 24A

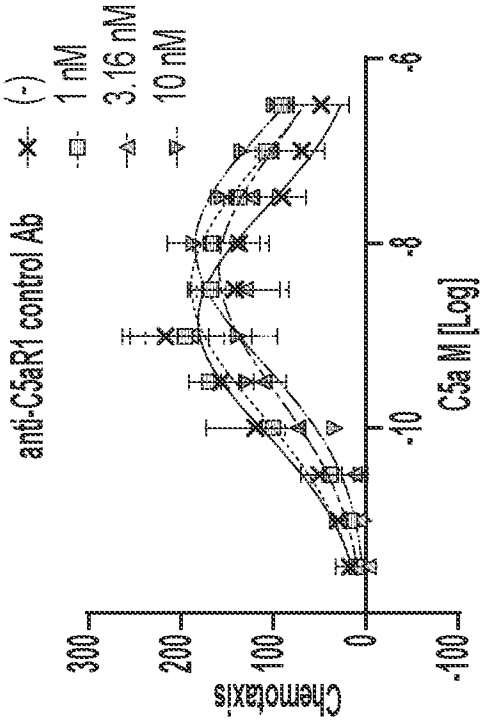
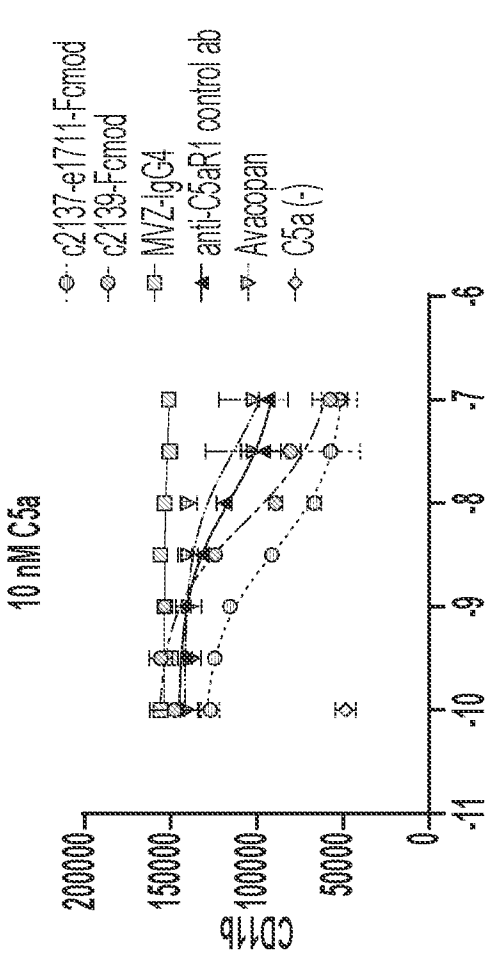
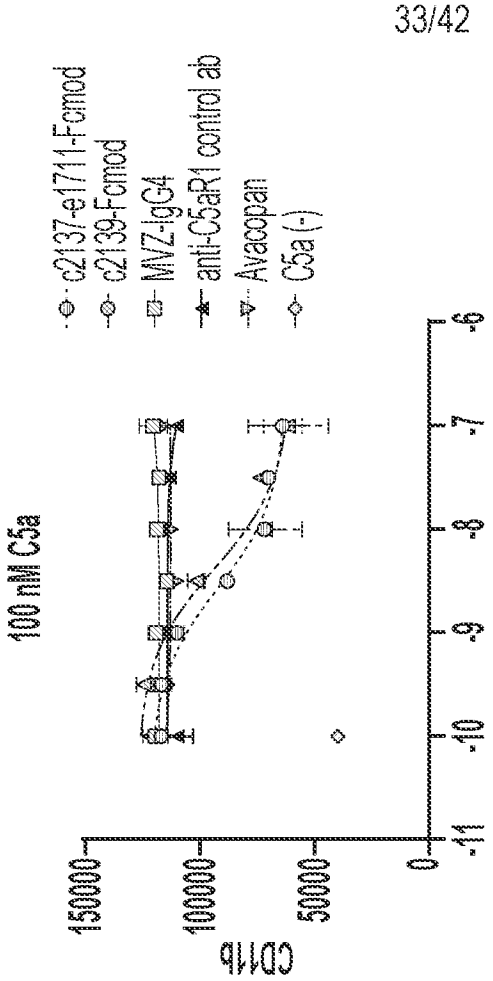


FIG. 24C



Antagonist	IC50 (nM) at 10 nM C5a
c2139	6.9
c2137-e1711	3.4
Avacopan	10.6
anti-C5aR1 control ab	33.1

FIG. 25A



Antagonist	IC50 (nM) at 100 nM C5a
c2139	4.2
c2137-e1711	2.7
Avacopan	Cannot be calculated
anti-C5aR1 control ab	Cannot be calculated

FIG. 25B

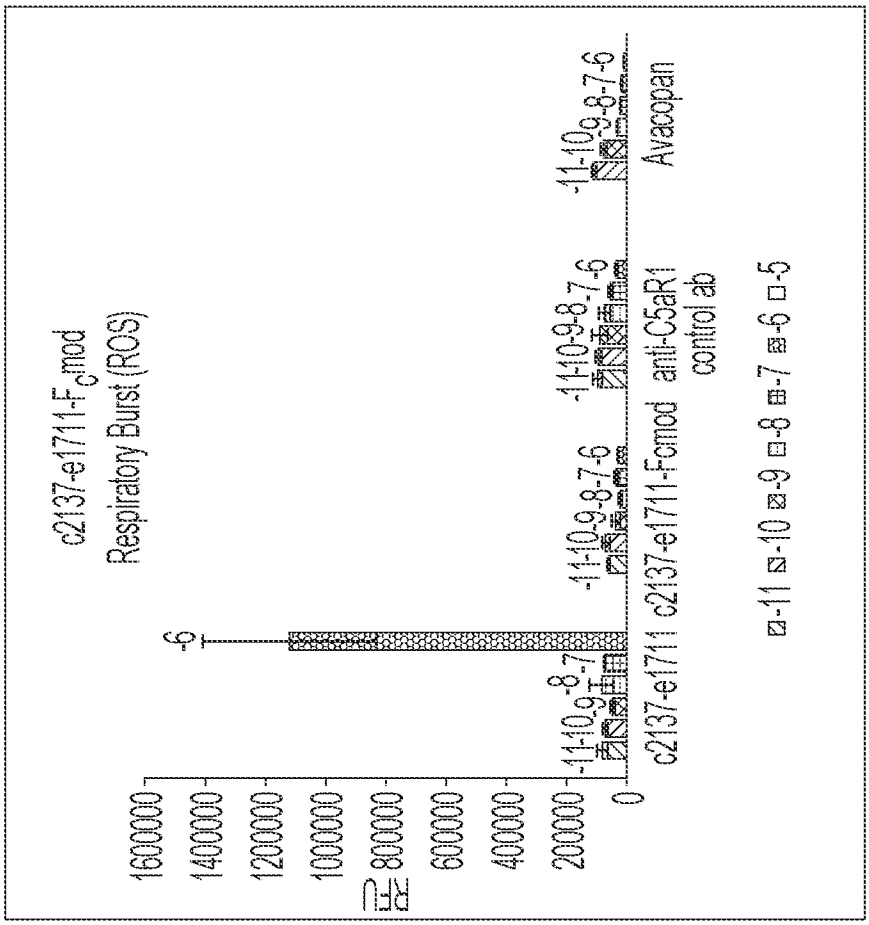


FIG. 26B

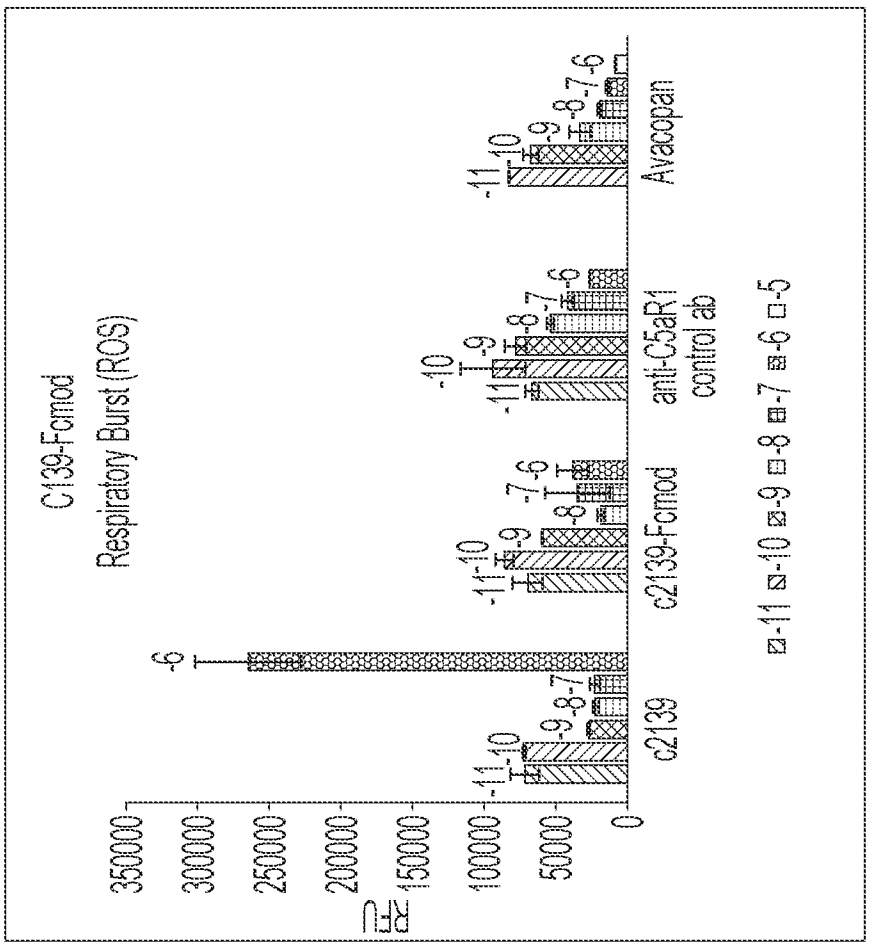
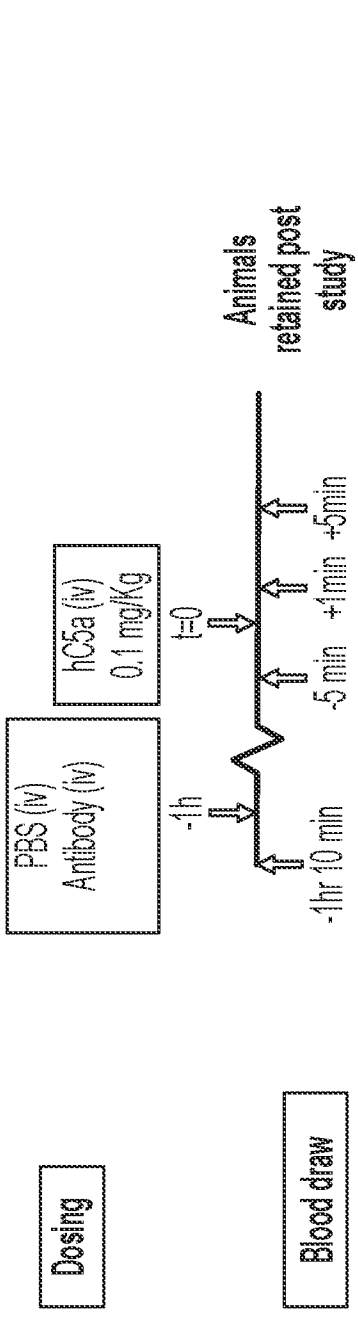


FIG. 26A



Group	N	Dose Route	Test Article	Dose (mg/kg)
2	5	IV	c2139-$F_c$mod	5
4	5	IV	c2137-e1711-$F_c$mod	5
5	5	IV	c2139	5
6	5	IV	c2137-e1711	5
7	5	IV	Vehicle	0
Endpoints	Blood collection: Pre-TA, Pre-C5a Dose, Post-C5a Dose up to 2 hr FACS analysis for Markers 7AAD, CD45, CD11b, Ly6G+, mCD88, hCD88			

FIG. 27A

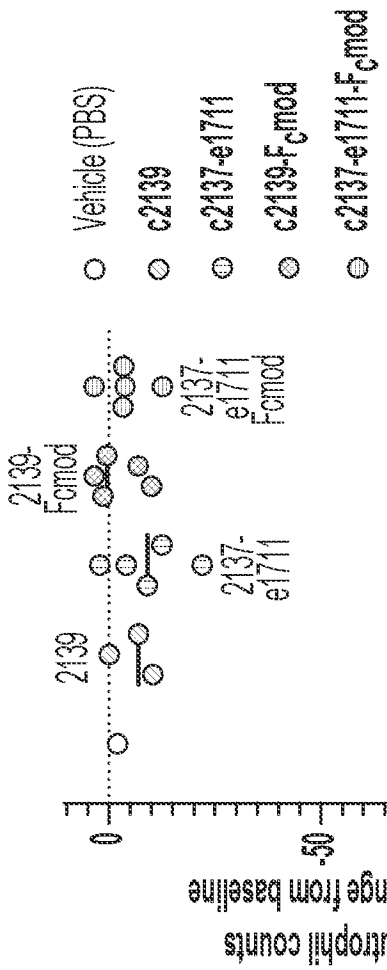


FIG. 27C

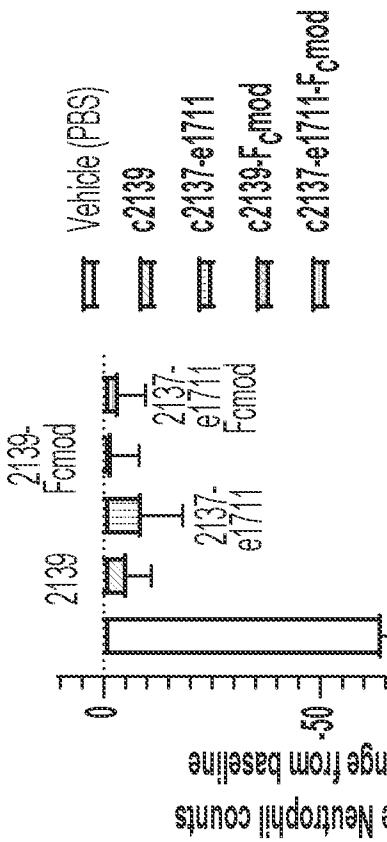


FIG. 27B

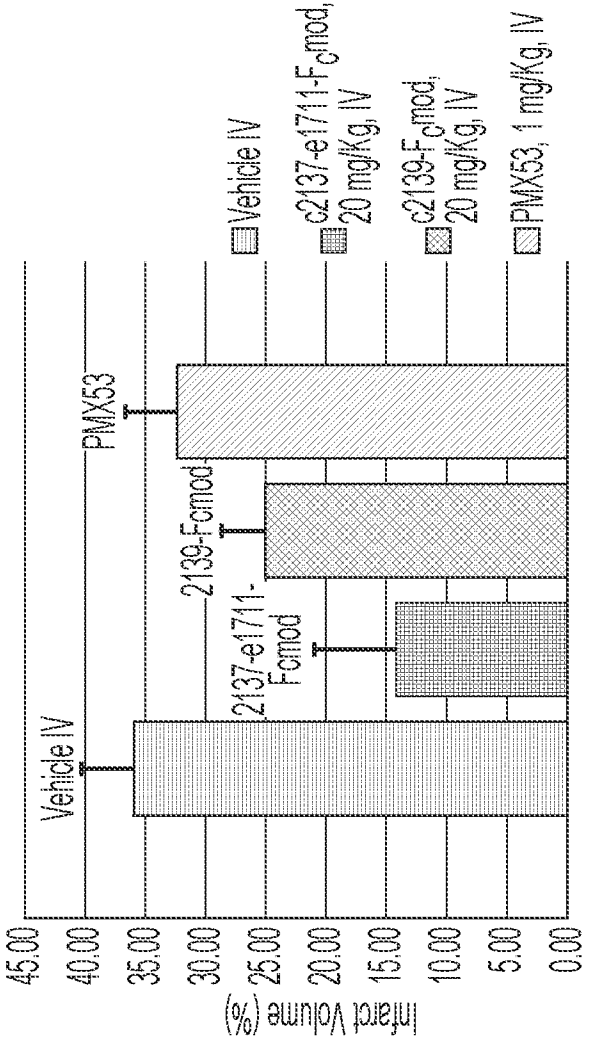


FIG. 28B

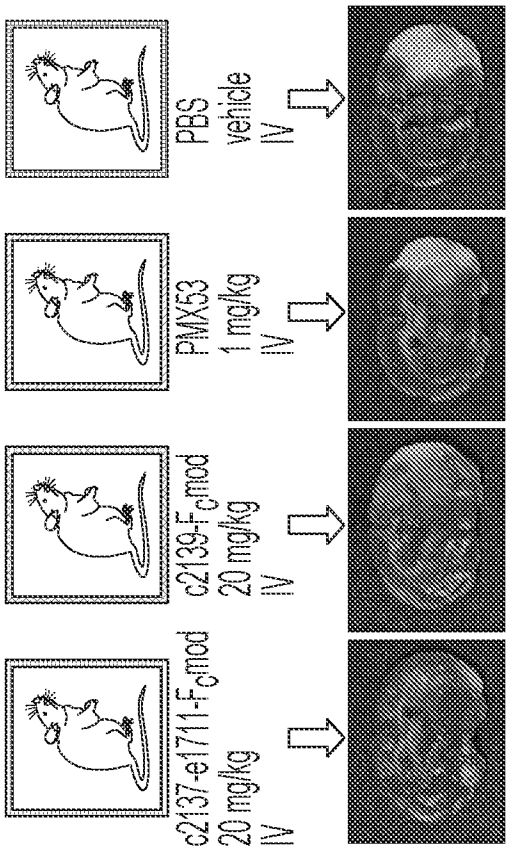


FIG. 28A

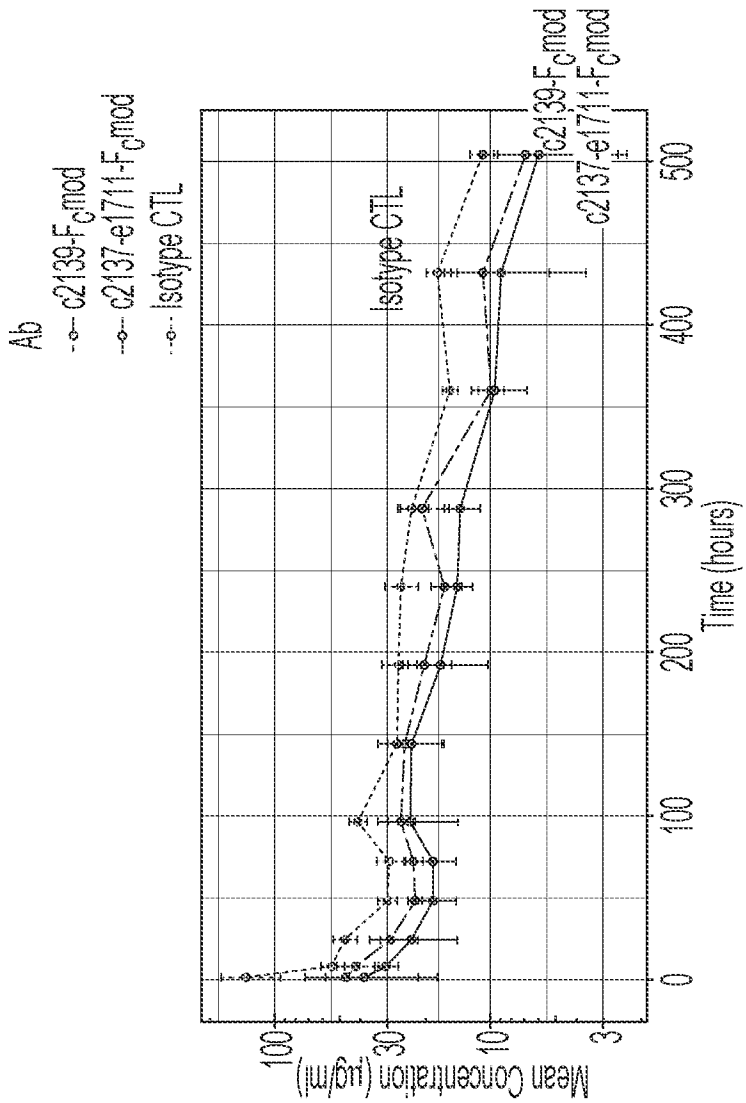


FIG. 29B

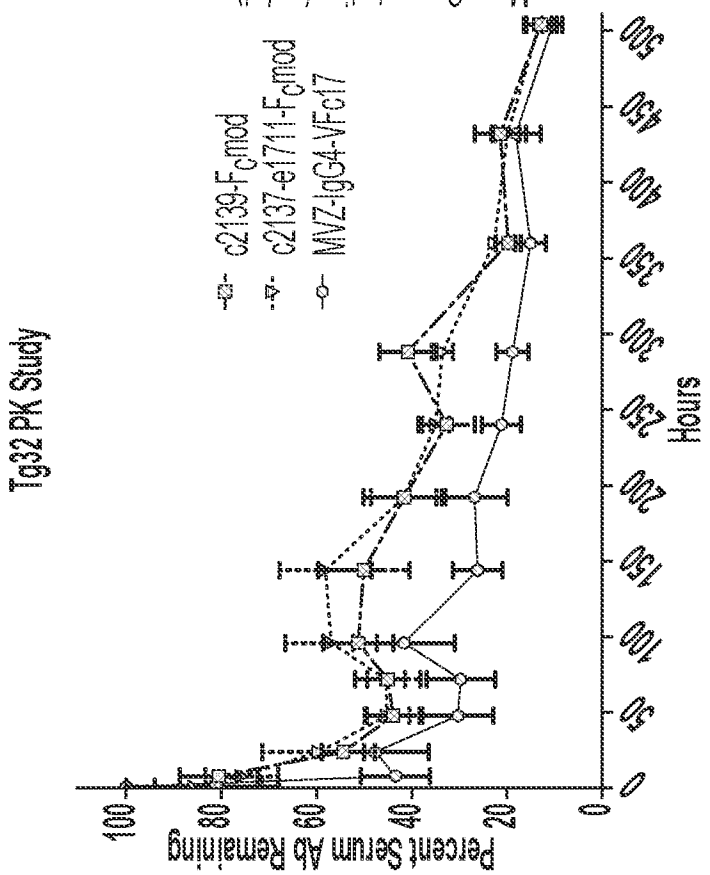


FIG. 29A

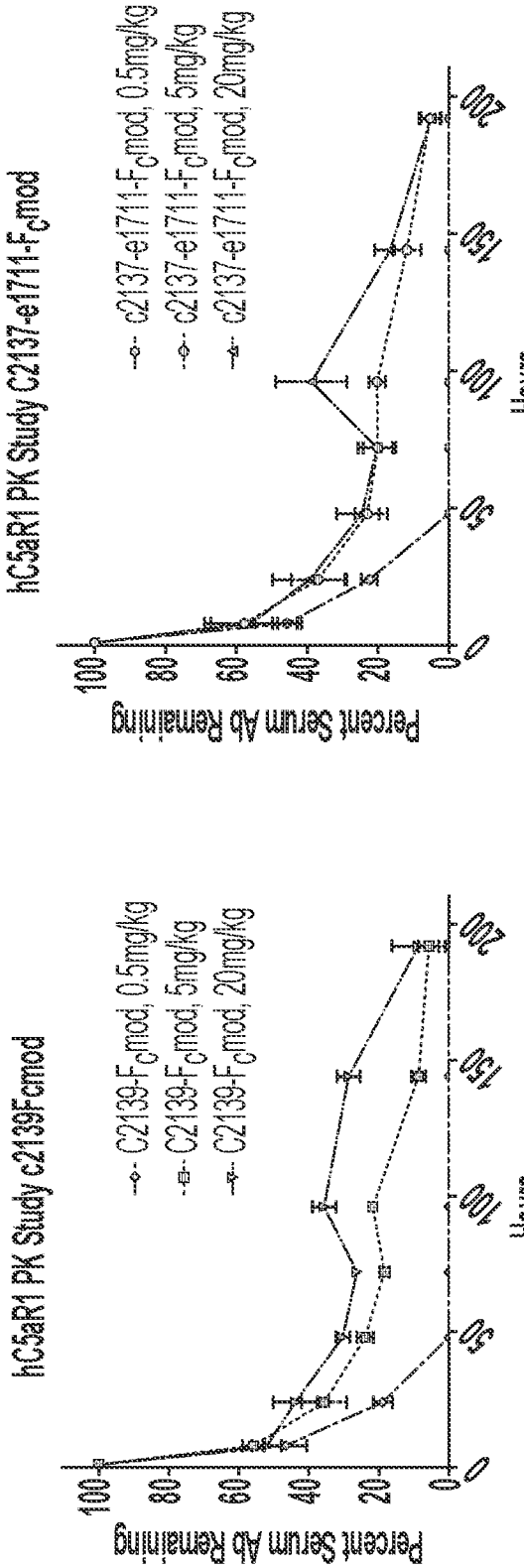


FIG. 30A

FIG. 30B

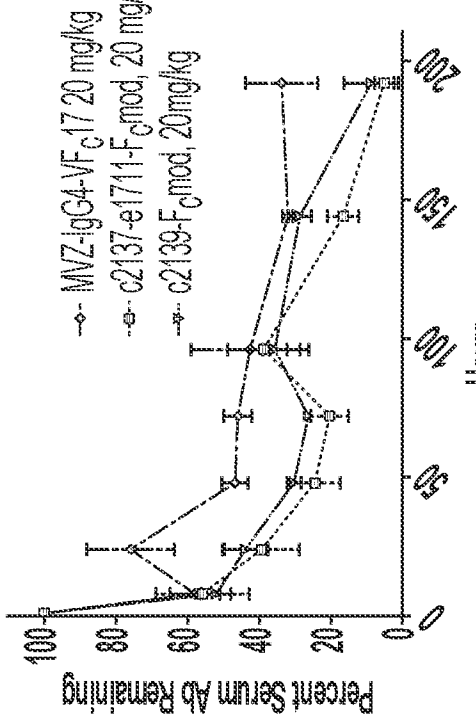


FIG. 30C

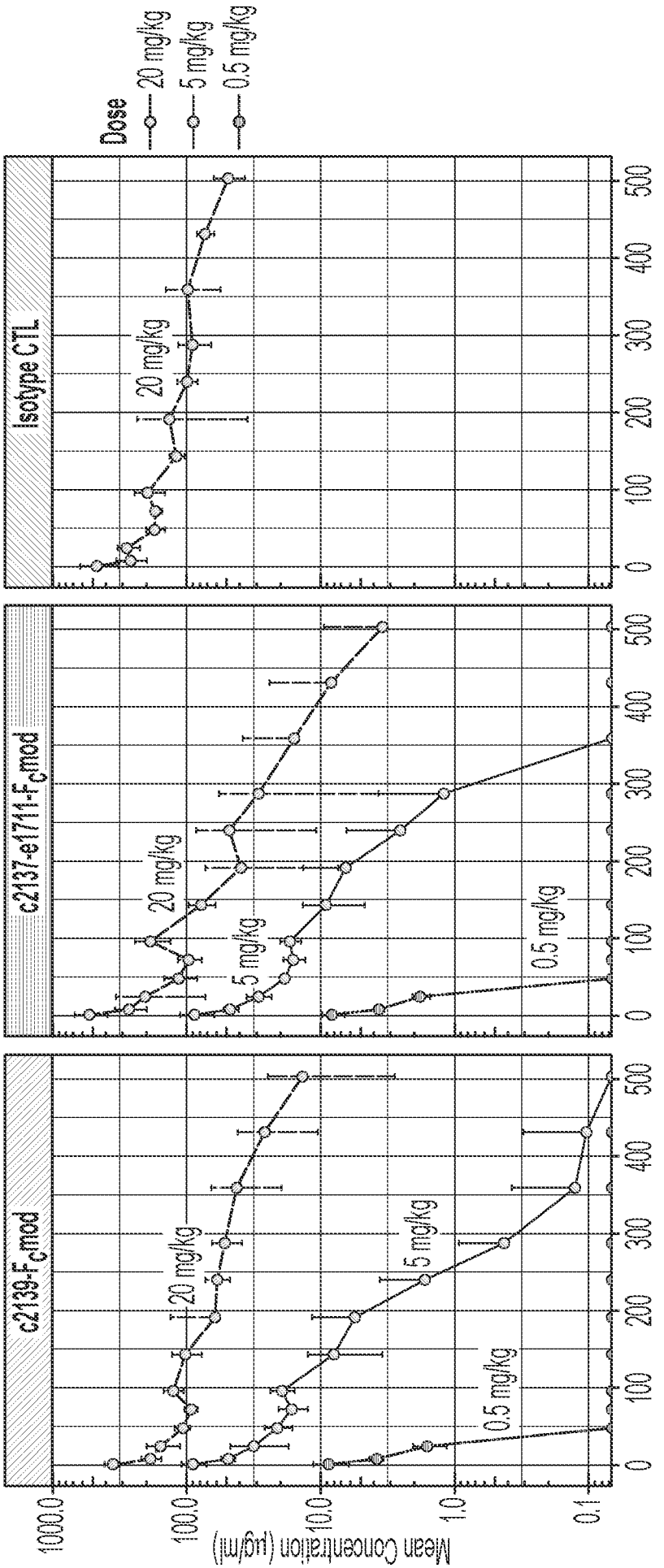


FIG. 30D

FIG. 30E

FIG. 30F

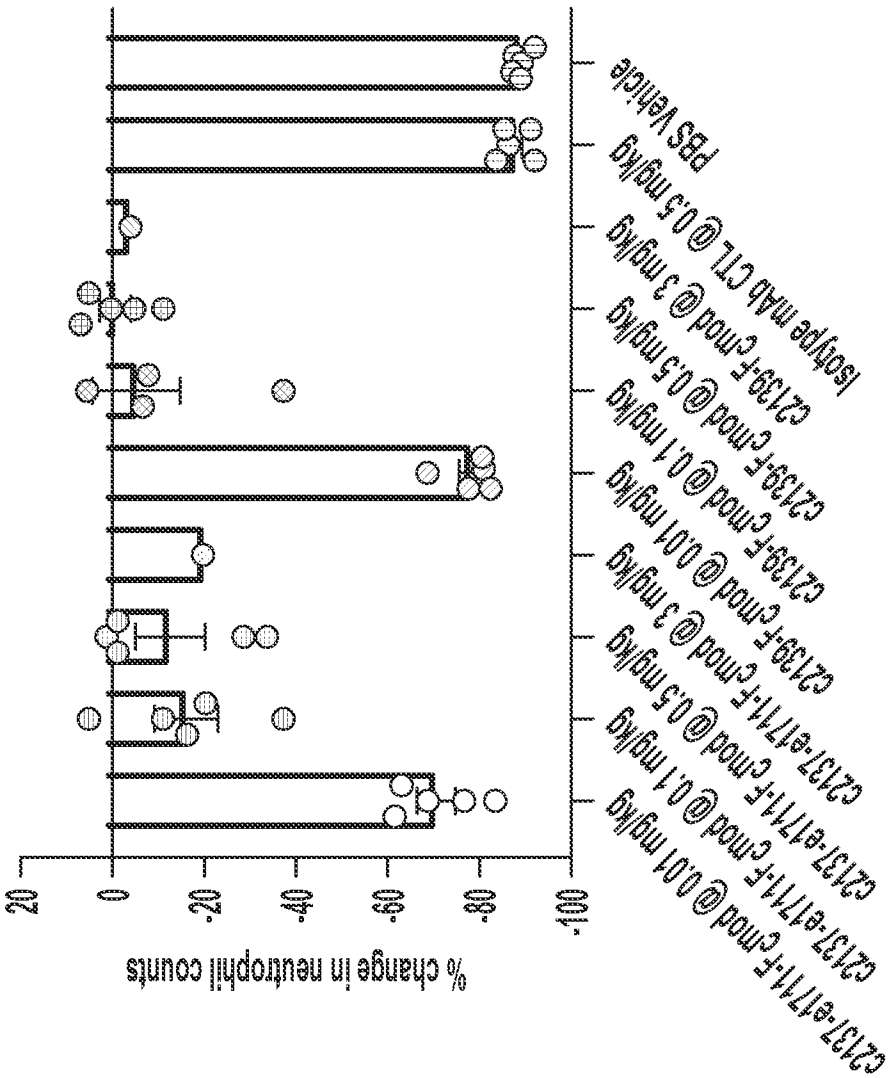

% change in neutrophil counts
20
0
-20
-40
-60
-80
-100
PBS Vehicle
Isotype mAb CTL @ 0.5 mg/kg
c2139-FcMod @ 3 mg/kg
c2139-FcMod @ 0.5 mg/kg
c2139-FcMod @ 0.1 mg/kg
c2139-FcMod @ 0.01 mg/kg
c2137-a77A1-FcMod @ 3 mg/kg
c2137-a77A1-FcMod @ 0.5 mg/kg
c2137-a77A1-FcMod @ 0.1 mg/kg
c2137-a77A1-FcMod @ 0.01 mg/kg

FIG. 31

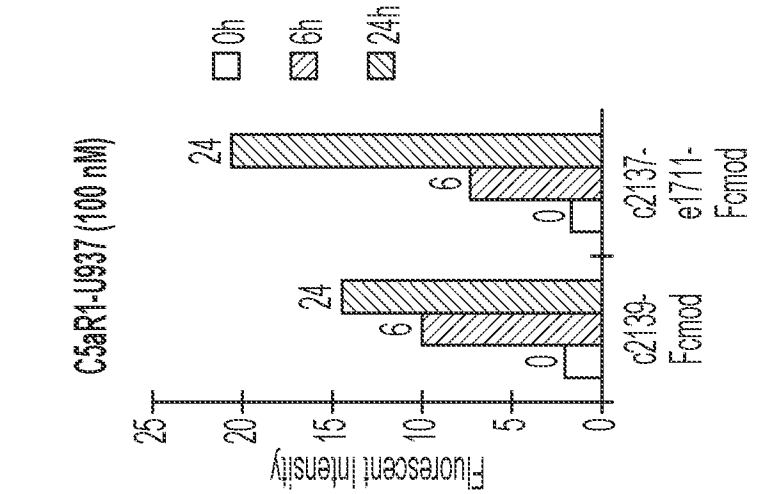


FIG. 32B

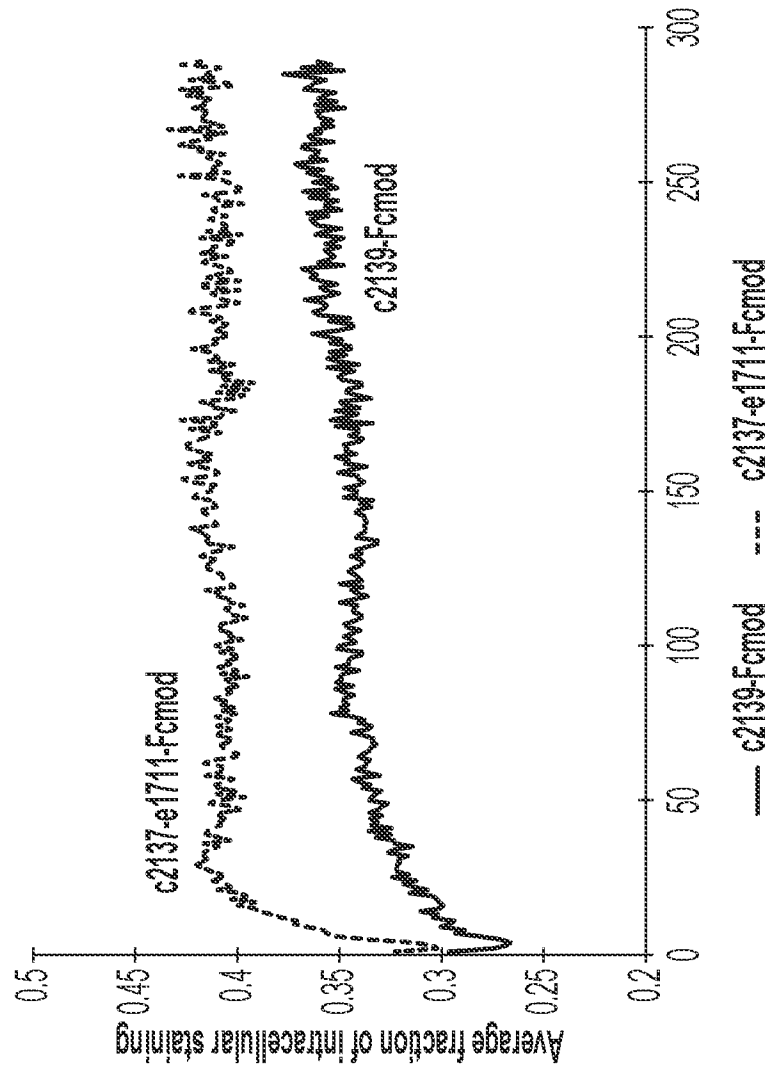


FIG. 32A

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/012317

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/28 A61P37/00 A61K39/395
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K A61P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, BIOSIS, CHEM ABS Data, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 2020/182974 A1 (MORPHOSYS AG [DE]) 17 September 2020 (2020-09-17) pages 3, 64-65 examples, in particular example 7; table 12 -----	1-38 81-98, 106,107
Y	WO 2012/168199 A1 (NOVO NORDISK AS [DK]; ZAHN STEFAN [DK] ET AL.) 13 December 2012 (2012-12-13) pages 3-4, 28-29; examples, in particular example 4; tables 2A, 2B -----	1-38, 81-98, 106,107
Y	WO 2009/103113 A1 (G2 INFLAMMATION PTY LTD [AU]; WHITFELD PETER [AU] ET AL.) 27 August 2009 (2009-08-27) pages 3-12; examples, in particular example 11 ----- -/--	1-38, 81-98, 106,107

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance;; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance;; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 April 2022	17/06/2022

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Bernhardt, Wiebke

1

Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2022/012317

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LILI HUANG ET AL: "Discovery of human antibodies against the C5aR target using phage display technology", JOURNAL OF MOLECULAR RECOGNITION, HEYDEN & SON LTD., LONDON, GB, vol. 18, no. 4, 1 June 2005 (2005-06-01), pages 327-333, XP002656207, ISSN: 0952-3499, DOI: 10.1002/JMR.735 [retrieved on 2005-02-10] abstract, figures	1-38, 81-98, 106,107
Y	US 2004/132101 A1 (LAZAR GREGORY ALAN [US] ET AL) 8 July 2004 (2004-07-08) page 67, table 61	81-98, 106,107
A	RUDIKOFF S ET AL: "Single amino acid substitution altering antigen-binding specificity", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, vol. 79, no. 6, 1 March 1982 (1982-03-01), pages 1979-1983, XP002683593, ISSN: 0027-8424, DOI: 10.1073/PNAS.79.6.1979 abstract	1-38, 81-98, 106,107
X,P	WO 2021/146320 A1 (VISTERRA INC [US]; VISWANATHAN KARTHIK [US] ET AL.) 22 July 2021 (2021-07-22)	1-38,81
Y,P	examples, claims; Seq ID No. 492, 582, 972, 1032	82-98, 106,107
A	ORT MARION ET AL: "Treatment of Rare Inflammatory Kidney Diseases: Drugs Targeting the Terminal Complement Pathway", FRONTIERS IN IMMUNOLOGY, vol. 11, 10 December 2020 (2020-12-10), XP055858384, DOI: 10.3389/fimmu.2020.599417 Retrieved from the Internet: URL:https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7758461/pdf/fimmu-11-599417.pdf> abstract, pages 13-14, figure 1, table 1	1-38, 81-98, 106,107

1

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/012317

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. [ ] forming part of the international application as filed:

      [ ] in the form of an Annex C/ST.25 text file.

      [ ] on paper or in the form of an image file.

   b. [ ] furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. [X] furnished subsequent to the international filing date for the purposes of international search only:

      [X] in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).

      [ ] on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).

2. [ ] In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2022/012317

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims;; it is covered by claims Nos.:

1-38, 96-98, 106, 107(completely); 81-95(partially)

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-38, 96-98, 106, 107(completely); 81-95(partially)

An antibody or antigen-binding fragment binding to complement component 5A receptor 1 (C5aR1) comprising the HCDRs with Seq ID No. 6-8 and the LCDRs with Seq ID No. 9-11; nucleic acid encoding said antibody; a cell comprising said nucleic acid; medical use thereof

---

2. claims: 39-80, 99-103(completely); 81-95(partially)

A biparatopic antibody or antigen-binding fragment comprising one antigen binding domain binding to C5aR1 at Seq ID No. 3 and one antigen-binding domain binding to C5aR1 at Seq ID No. 1 or Seq ID No. 2; nucleic acid encoding said antibody; a cell comprising said nucleic acid; medical use thereof

---

3. claims: 104, 105

An antibody or antigen-binding fragment binding to complement component 5A receptor 1 (C5aR1) comprising VH with Seq ID No. 43 and VL with Seq ID No. 48.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/012317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2020182974 A1	17-09-2020	AU 2020235263 A1	02-09-2021
		CA 3132771 A1	17-09-2020
		CN 113574072 A	29-10-2021
		EP 3938393 A1	19-01-2022
		JP 2022524449 A	02-05-2022
		KR 20210141988 A	23-11-2021
		SG 11202108761Y A	29-09-2021
		TW 202041535 A	16-11-2020
		US 2022135658 A1	05-05-2022
		WO 2020182974 A1	17-09-2020
WO 2012168199 A1	13-12-2012	AU 2012266487 A1	21-11-2013
		BR 112013031198 A2	29-11-2016
		CA 2838497 A1	13-12-2012
		CN 103596982 A	19-02-2014
		DK 2718322 T3	03-12-2018
		DK 3424953 T3	02-11-2020
		EP 2718322 A1	16-04-2014
		EP 3424953 A1	09-01-2019
		EP 3798230 A1	31-03-2021
		ES 2693647 T3	13-12-2018
		IL 229254 A	28-09-2017
		JP 6141834 B2	07-06-2017
		JP 6416177 B2	31-10-2018
		JP 2014523408 A	11-09-2014
		JP 2017081941 A	18-05-2017
		KR 20140036261 A	25-03-2014
		PL 2718322 T3	31-07-2019
		PL 3424953 T3	25-01-2021
		PT 3424953 T	03-11-2020
		RU 2013155029 A	20-07-2015
		TW 201311724 A	16-03-2013
		US 2013004514 A1	03-01-2013
		US 2013295116 A1	07-11-2013
		US 2015044231 A1	12-02-2015
		US 2017073421 A1	16-03-2017
		US 2020017599 A1	16-01-2020
		US 2021238300 A1	05-08-2021
		WO 2012168199 A1	13-12-2012
WO 2009103113 A1	27-08-2009	AU 2009217224 A1	27-08-2009
		BR PI0907532 A2	28-07-2015
		CA 2714296 A1	27-08-2009
		CN 101970494 A	09-02-2011
		CN 104193824 A	10-12-2014
		EP 2254911 A1	01-12-2010
		EP 2826791 A2	21-01-2015
		ES 2562627 T3	07-03-2016
		IL 207217 A	31-03-2015
		JP 6034826 B2	30-11-2016
		JP 2011515073 A	19-05-2011
		JP 2014193164 A	09-10-2014
		KR 20100117120 A	02-11-2010
		RU 2010138612 A	27-03-2012
		US 2011190477 A1	04-08-2011
		US 2012315275 A1	13-12-2012
		US 2013129721 A1	23-05-2013
		WO 2009103113 A1	27-08-2009

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2022/012317

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
		ZA 201005348 B	26-10-2011
US 2004132101 A1	08-07-2004	AU 2004236160 A1	18-11-2004
		AU 2008261120 A1	15-01-2009
		BR PI0410031 A	25-04-2006
		BR 122018016045 B1	13-10-2020
		CA 2524399 A1	18-11-2004
		CA 2916863 A1	18-11-2004
		CN 1867583 A	22-11-2006
		CN 102633880 A	15-08-2012
		CN 104788565 A	22-07-2015
		DK 2368911 T3	11-09-2017
		EP 1620467 A2	01-02-2006
		EP 2368911 A1	28-09-2011
		EP 3101030 A1	07-12-2016
		EP 3838920 A1	23-06-2021
		ES 2638568 T3	23-10-2017
		HU E034268 T2	28-02-2018
		IL 171723 A	31-03-2014
		IL 265538 A	31-05-2020
		JP 4578467 B2	10-11-2010
		JP 2007525443 A	06-09-2007
		KR 20050116400 A	12-12-2005
		KR 20070116176 A	06-12-2007
		PL 2368911 T3	29-12-2017
		SI 2368911 T1	30-10-2017
		US 2004132101 A1	08-07-2004
		US 2009068177 A1	12-03-2009
		US 2009081208 A1	26-03-2009
		US 2009092599 A1	09-04-2009
		US 2012230980 A1	13-09-2012
		US 2013156754 A1	20-06-2013
		US 2013156758 A1	20-06-2013
		US 2013243762 A1	19-09-2013
		US 2015079082 A1	19-03-2015
		US 2016318993 A1	03-11-2016
		US 2016347825 A1	01-12-2016
		US 2016347837 A1	01-12-2016
		WO 2004099249 A2	18-11-2004
WO 2021146320 A1	22-07-2021	TW 202140553 A	01-11-2021
		US 2022089764 A1	24-03-2022
		WO 2021146320 A1	22-07-2021