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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
29 October 2020 (29.10.2020)

WIPO | PCT

(10) International Publication Number
WO 2020/219681 A1

(51) International Patent Classification:
A61K 39/395 (2006.01) *A61P 35/00* (2006.01) *C07K 16/28* (2006.01) *A61K 39/00* (2006.01)

(21) International Application Number: PCT/US2020/029531

(22) International Filing Date: 23 April 2020 (23.04.2020)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

62/837,518	23 April 2019 (23.04.2019)	US
62/859,699	10 June 2019 (10.06.2019)	US
20305145.3	17 February 2020 (17.02.2020)	EP
20305146.1	17 February 2020 (17.02.2020)	EP

(71) Applicants: **SANOFI** [FR/FR]; 54 rue La Boétie, 75008 Paris (FR). **GENZYME CORPORATION** [US/US]; 50 Binney Street, Cambridge, Massachusetts 02142 (US).

(72) Inventors: **CAMERON, Béatrice**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **CHIRON BLONDEL, Marielle**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **DUMAS, Jacques**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **FOURNIER, Alain**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **KINGSBURY, Jonathan**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-525, Bridgewater, New Jersey 08807 (US). **LEMOINE, Cendrine**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **MURRAY, Brian**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-525, Bridgewater, New Jersey 08807 (US). **OSTBERG, Nathan**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-525, Bridgewater, New Jersey 08807 (US). **PATKE, Sanket**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-525, Bridgewater, New Jersey 08807 (US). **VIRONE-ODDOS, Angela**; c/o Sanofi, 54 rue La Boétie, 75008 Paris (FR). **ZHANG, Zichuan**; c/o Sanofi, 55 Corporate Drive, Mail Code 55A-525, Bridgewater, New Jersey 08807 (US).

(74) Agent: **VELEMA, James H.**; 28 State Street, Boston, Massachusetts 02109 (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, 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, 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))*
— *with sequence listing part of description (Rule 5.2(a))*

WO 2020/219681 A1

(54) **Title:** ANTI-CD38 ANTIBODIES AND FORMULATIONS

(57) **Abstract:** Provided herein are antibodies that specifically bind human CD38, formulations and unit dosage forms comprising the antibodies, methods of preparing the antibodies and methods of using the antibodies.

ANTI-CD38 ANTIBODIES AND FORMULATIONS

RELATED APPLICATIONS

This application claims benefit of United States Provisional Application No. 62/837,518, filed April 23, 2019; United States Provisional Application No. 62/859,699, filed June 10, 2019; European Patent Application No. 20305145.3, filed February 17, 2020; and European Patent Application No. 20305146.6, filed February 17, 2020, the entire contents of which are incorporated herein by reference.

SEQUENCE LISTING

The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on April 17, 2020, is named 704023_SA9-289PC_ST25.txt and is 44,783 bytes in size.

FIELD OF THE INVENTION

[0001] Provided herein are anti-CD38 antibodies having improved cytotoxic activity, and stable formulations thereof.

BACKGROUND OF THE INVENTION

[0002] CD38 is a Type II glycosylated 45 kilodalton (kDa) membrane protein that was identified as a lymphocyte marker. CD38 has a role in leukocyte homeostasis through modulation of hematopoietic cell survival and differentiation (Richards JO, et al., Mol Cancer Ther. 2008; 7(8):2517-27). CD38 functions as a receptor binding to CD31 and is involved in cell adhesion and signal transduction. The function of CD38 in signal transduction appears to be versatile depending on the cell lineage, the differentiation stage, and, possibly, the association with different co-receptors (Richards JO, et al., 2008). CD38 is also an ectoenzyme catalyzing the synthesis and hydrolysis of cyclic adenosine-diphosphate-ribose (cADPR) from nicotinamide adenine dinucleotide (NAD+) to ADP-ribose (DiLillo DJ, Ravetch JV., Cell. 2015; 161(5):1035-45). These reaction products are implicated in calcium mobilization and intracellular signaling (Derer S, et al., MAbs. 2014; 6(2):409-21).

[0003] The expression of CD38 in healthy humans can be detected on NK cells, monocytes, dendritic cells, macrophages, granulocytes, activated T and B cells, and plasma cells. In contrast, expression has not been detected in hematopoietic stem cells, resting T and

B cells, or tissue macrophages. Furthermore, several hematological malignancies express CD38, such as plasma cell dyscrasias (e.g., multiple myeloma (MM), amyloidosis) and other cancers of hematopoietic origin including, including for example, Waldenstrom's disease, non-Hodgkin's lymphoma (NHL), acute lymphocytic leukemia (ALL), and acute myelogenous leukemia (AML).

[0004] The expression of CD38 is especially notable in MM as >98% of patients are positive for this protein (Reinherz EL, et al., Proc Natl Acad Sci USA. 1980; 77(3):1588-92, Lin P, et al., Am J Clin Pathol. 2004; 121(4):482-8). The strong and uniform expression of CD38 on malignant clonal MM cells contrasts with the restricted expression pattern on normal cells suggesting this antigen may be useful for specific targeting of tumor cells.

[0005] MM is a malignant plasma cell disease that is characterized by CD38 expression on the cell surface, clonal proliferation of plasma cells in the bone marrow (BM) and the production of excessive amounts of a monoclonal immunoglobulin (usually of the IgG or IgA type or free urinary light chain (also referred to as paraprotein, M-protein or M-component)). It is a disease predominantly associated with advancing age with more than 80% of patients aged 60 years or older.

[0006] The disease course for MM varies with the aggressiveness of the disease and related prognostic factors. Certain chromosomal abnormalities in multiple myeloma have been shown to be associated with poor clinical outcome. High-risk cytogenetic changes include del(17p), t(4;14),and t(14;16) and 1q gain, among others. During the two last decades, median survival has improved from 3 to 6 years; however, some patients can live longer than 10 years (Ocio EM, et al., Expert Rev Hematol. 2014; 7(1):127-41). Treatment options and survival are based on the patient's age, fitness and disease status. Patients under the age of approximately 65, presenting with symptomatic active disease in good physical health will generally receive initial therapy with autologous stem cell transplantation (ASCT). To achieve cytoreduction of the disease before collecting stem cells, induction chemotherapy is administered. Induction treatment regimens include alkylating agents, dexamethasone alone, thalidomide plus dexamethasone, and vincristine, Adriamycin® (doxorubicin), and dexamethasone (VAD; or modifications to this regimen); however, the latter two regimens are associated with higher toxicity (Richardson PG, et al., Blood. 2010; 116(5):679-86, Arnulf B, et al., Haematologica. 2012; 97:1925-8). Treatments with Velcade® (bortezomib) alone, bortezomib combinations, and Revlimid® (lenalidomide) plus dexamethasone have demonstrated improved outcomes as induction therapy, and these agents demonstrate higher response rates and lower toxicity (Richardson PG 2010, Kumar S, et al., Blood. 2012;

119(19):4375-82; Roussel M, et al., J Clin Oncol. 2014; 32:2712-7; Durie BGM, et al., Lancet. 2017; 389:519-27). Additional approved drugs include pomalidomide (belonging to the same IMiD® class as lenalidomide) and carfilzomib and ixazomib (belonging to the same proteasome inhibitor class as bortezomib). In addition to these new treatments, monoclonal antibodies, particularly anti-CD38 antibodies, have begun to play a major role in the treatment of myeloma patients. Daratumumab, an anti-CD38 antibody,has been approved for treatment of MM as a single agent and in combination with other treatments for MM (Touzeau C, Moreau P., Expert Opin Biol Ther. 2017; 17(7):887-93; Tzogani K., et al., Oncologist. 2018; 23:1-11). Isatuximab, an anti-CD38 antibody, has been reported to induce 25-29% response rate as a single agent and 60% response rate in combination therapy in relapsed or refractory multiple myeloma (Martin T, et al., Blood. 2017; 129(25):3294-303); recent results of phase 3 study indicated that the addition of isatuximab is able to improve the progression-free survival with pomalidomide-dexamethasone in relapsed and refractory multiple myeloma (J Clin Oncol 37, 2019 (suppl; abstr 8004)). In addition, elotuzumab, an anti-Slam-F7 antibody, has been approved in combination with lenalidomide and dexamethasone for treatment of adult patients with MM who have received one to three prior lines of therapy, and in combination with pomalidomide and dexamethasone for the treatment of adult patients with MM who have received at least two prior therapies including lenalidomide and a proteasome inhibitor (Bristol-Myers Squibb Company. EMPLICITI® (elotuzumab) [package insert]. U.S. Food and Drug Administration website. www-dot-accessdata-dot-fda.gov/drugsatfda_docs/label/2018/761035s008lbl.pdf. Revised November 2018.

[0007] The current aim of therapy for these CD38-expessing diseases is to control the disease as effectively as possible, to maximize quality of life and to prolong survival. For example, MM patients will receive an average of 4 to 8 different regimens during their lifespan. Thus, despite the improvement in patient outcomes with newer therapies, MM a remains fatal disease. Thus, the treatment of patients who have received and progressed on current therapies, including antibody therapies, remains a significant challenging unmet medical need.

SUMMARY OF THE INVENTION

[0008] Compared to currently available treatments for CD38-expressing diseases, including antibody treatments, it is contemplated that the antibodies, uses, and methods of treatment provided herein may provide a superior clinical response or treatment of the disease. Provided herein are antibodies that specifically bind human CD38 and mediate superior killing of cells that express CD38, formulations and unit dosage forms comprising the antibodies, methods of preparing the antibodies, and methods of using the antibodies.

[0009] In one aspect, antibodies that specifically bind human CD38 are provided. In some embodiments, the antibodies provided herein comprise a light chain (LC) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 8, and 9 and a heavy chain (HC) having an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 3, 4, 5, and 6.

[0010] In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 7 and an HC having an amino acid sequence selected from the group consisting of: SEQ ID NO: 2, SEQ ID NO: 3, and SEQ ID NO: 4. In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 7 and an HC having an amino acid sequence of SEQ ID NO: 2. In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 7 and an HC having an amino acid sequence of SEQ ID NO: 3. In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 7 and an HC having an amino acid sequence of SEQ ID NO: 4.

[0011] In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 8 and an HC having an amino acid sequence selected from the group consisting of SEQ ID NO: 5 and SEQ ID NO: 6. In some embodiments, the antibodies provided herein comprise and LC having an amino acid sequence of SEQ ID NO: 8 and HC having an amino acid sequence of SEQ ID NO: 5. In some embodiments, the antibodies provided herein comprise and LC having an amino acid sequence of SEQ ID NO: 8 and HC having an amino acid sequence of SEQ ID NO: 6.

[0012] In some embodiments, the antibodies provided herein comprise an LC having an amino acid sequence of SEQ ID NO: 9 and an HC having an amino acid sequence selected from the group consisting of SEQ ID NO: 6 and SEQ ID NO: 7. In some embodiments, the antibodies provided herein comprise and LC having an amino acid sequence of SEQ ID NO: 9 and HC having an amino acid sequence of SEQ ID NO: 6. In some embodiments, the antibodies provided herein comprise and LC having an amino acid sequence of SEQ ID NO: 9 and HC having an amino acid sequence of SEQ ID NO: 7.

[0013] In another aspect, pharmaceutical compositions comprising a formulated antibody are provided herein. In some embodiments of the pharmaceutical compositions, the antibody comprises HCs and LCs having the amino acid sequences as described above, in combination with sucrose, L-histidine, and polysorbate 80. In some embodiments, the antibody is present at a concentration of 50 mg/mL, the sucrose is present at a concentration of 8 % (w/v), the L-histidine is present at a concentration of 10 mM, the polysorbate 80 (PS80) is present at a concentration of 0.05 % (v/v), and the formulation has a pH of 6.2. In some embodiments, the pharmaceutical composition is lyophilized.

[0014] In another aspect, unit dosage forms comprising a formulated antibody are provided herein. In some embodiments, the antibody comprises HCs and LCs having the amino acid sequences as described above and the unit dosage form comprises 215 mg of the antibody, 6.21 mg L-histidine, 344 mg sucrose, and 2.15 mg polysorbate 80. In some embodiments, the unit dosage form of the antibody is lyophilized.

[0015] In another aspect, processes for preparing pharmaceutical compositions comprising an antibody that specifically binds human CD38 are provided herein. In some embodiments, the antibody comprises HCs and LCs having the amino acid sequences as described above, wherein the process comprises expressing the antibody in a cell culture, subjecting the antibody to at least one of a chromatography purification step and an ultrafiltration step to produce a purified antibody solution; and adjusting the purified antibody solution to produce an antibody formulation. In some embodiments, the antibody formulation comprises the purified antibody, sucrose, L-histidine, and polysorbate 80. In some embodiments, antibody formulation comprises the antibody at a concentration of 50 mg/mL, sucrose at a concentration of 8 % w/v, L-histidine at a concentration of 10 mM; and polysorbate 80 (PS80) at a concentration of 0.05 % v/v. In some embodiments, the antibody formulation is prepared such that it has a pH of 6.2. In some embodiments of the process for preparing the antibody formulation, the antibody formulation is lyophilized.

[0016] In another aspect, methods for preparing a reconstituted antibody formulation are provided. In some embodiments, the lyophilized antibody formulation comprises sucrose, L-histidine, PS80, and an antibody that specifically binds human CD38. In some embodiments, the antibody comprises HCs and LCs having the amino acid sequences as described above. In some embodiments, the lyophilized antibody formulation is reconstituted in a diluent, thereby preparing the reconstituted antibody formulation. In some embodiments, the reconstituted antibody formulation comprises the antibody at a concentration of 50 mg/mL, sucrose at a concentration of 8 % w/v, L-histidine at a concentration of 10 mM; and PS80 at a concentration of 0.05 % v/v. In some embodiments, the reconstituted antibody formulation has a pH of 6.2.

[0017] In another aspect, methods of treating a patient having a Multiple Myeloma are provided. In some embodiments, the method comprises administering to the patient one or more doses of an antibody that specifically binds human CD38, wherein the antibody comprises HCs and LCs having the amino acid sequences as described above.

[0018] In another aspect, antibodies for use in a method of treating Multiple Myeloma are provided. In some embodiments, the antibody specifically binds human CD38, wherein the antibody comprises HCs and LCs having the amino acid sequences as described above.

BRIEF DESCRIPTION OF THE DRAWINGS

[0019] FIG. 1 is a graph showing the onset of unfolding for mAbs 1, 2, 3, 5, 6, 7, and 8.

[0020] FIG. 2 is a graph showing isoelectric point (pI) for mAbs 1, 3, 5, 6, 7, and 8.

DETAILED DESCRIPTION OF THE INVENTION

[0021] Provided herein are anti-CD38 antibodies that have superior antibody-dependent cellular cytotoxicity (ADCC) against cells expressing high, medium, and low levels of CD38 on their cell surfaces. In some embodiments, the anti-CD38 antibodies provided herein also have superior antibody-dependent cellular phagocytosis (ADCP) activity against cells expressing CD38 on their cell surfaces. In some embodiments, the antibodies provided herein have a lower than expected isoelectric point (pI). Despite the lower than expected pI and onset of protein unfolding at a lower temperature, which could negatively impact the stability of the antibody, especially in commercial production processes, the antibodies provided herein are provided in stable suitable form for administration to human patients.

Antibodies

[0022] Provided herein are anti-CD38 antibodies that specifically bind human CD38. The anti-CD38 antibodies provided herein may be produced using recombinant methods. For recombinant production of an anti-antigen antibody, nucleic acid encoding the antibody is isolated and inserted into a replicable vector for further cloning (amplification of the DNA) or for expression. DNA encoding the antibody may be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of the antibody). Many vectors are available. The vector components generally include, but are not limited to, one or more of the following: a signal sequence, an origin of replication, one or more marker genes, an enhancer element, a promoter, and a transcription termination sequence. The vector is typically transformed into a host cell suitable for expression of the nucleic acid. In some embodiments, the host cell is a eukaryotic cell or a prokaryotic cell. In some embodiments, the eukaryotic host cell is a mammalian cell. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al., J. Gen Virol. 36:59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23:243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL-1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, HB 8065); mouse mammary tumor (MMT 060562, ATCC CCL51); TRI cells (Mather et al., Annals N.Y. Acad. Sci. 383:44-68 (1982)); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2). Other useful mammalian host cell lines include Chinese hamster ovary (CHO) cells, including DHFR-CHO cells (Urlaub et al., Proc. Natl. Acad. Sci. USA 77:4216 (1980)); and myeloma cell lines such as NS0 and Sp2/0. For a review of certain mammalian host cell lines suitable for antibody production, see, e.g., Yazaki and Wu, Methods in Molecular Biology, Vol. 248 (B. K. C. Lo, ed., Humana Press, Totowa, N.J., 2003), pp. 255-268. The anti-CD38 antibody prepared from the cells can be purified using, for example, hydroxylapatite chromatography, hydrophobic interaction chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being among one of the typically preferred purification steps. In general, various methodologies for preparing antibodies for use in research, testing, and clinical applications are well-established in the art, consistent with the above-described methodologies and/or as deemed appropriate by one skilled in the art.

[0023] In some embodiments, the antibody is expressed by the CHO 8D6 host cell line (DXB11 derivative) using a 500 L single-use bioreactor, operated in fed-batch mode. The cell culture process is initiated by thawing a master cell bank vial, followed by a series of seed train cell expansion steps. The cells are then transferred into a bioreactor and grown using a serum free, chemically-defined cell culture medium. The culture is terminated for harvest of the antibodies after 10-14 days. The cells and cell culture debris can be removed from the harvested cell culture by depth filtration.

[0024] Harvested material is then further processed through chromatographic and filtration steps to produce purified antibody. The purified antibody is formulated in a liquid solution and is stored at ≤ -30° C.

[0025] Prior to dispensing the formulated antibody into suitable vials, the liquid solution is thawed and sterile filtered using a 0.2 μm filtration device. The formulated antibody is dispensed into a suitable container, such as a USP Type 1 glass vial, 4.3 mL/vial. In some embodiments, the filled vial includes a 0.3 mL overage of the formulated antibody. In some embodiments, the formulated antibody is lyophilized in the vial.

[0026] The term "antibody" typically refers to a tetrameric protein comprising two heavy chains (HC) and two light chains (LC). Each such tetramer is typically composed of two identical pairs of polypeptide chains, each pair having one LC (typically having a molecular weight of about 25 kDa) and one HC (typically having a molecular weight of about 50-70 kDa). The terms "HC" and "LC" as used herein refer to any immunoglobulin polypeptide having sufficient variable domain sequence to confer specificity for a target antigen. The amino-terminal portion of each light and heavy chain typically includes a variable domain of about 100 to 110 or more amino acids that typically is responsible for antigen recognition. The carboxy-terminal portion of each chain typically defines a constant domain responsible for effector function. Thus, in a typical antibody, a full-length HC immunoglobulin polypeptide includes a variable domain (VH) and three constant domains (CH1, CH2, and CH3), wherein the VH domain is at the amino-terminus of the polypeptide and the CH3 domain is at the carboxyl-terminus, and a full-length LC immunoglobulin polypeptide includes a variable domain (VL) and a constant domain (CL), wherein the VL domain is at the amino-terminus of the polypeptide and the CL domain is at the carboxyl-terminus.

[0027] The term "antibody" is used herein in the broadest sense and specifically covers typical antibodies, as described above, including monoclonal antibodies, and multispecific antibodies (e.g., bi and tri-specific antibodies so long as they exhibit the desired biological activity including specific binding to the CD38 target and ability to trigger ADCC and ADCP).

[0028] The term "Fc" as used herein refers to a molecule comprising the sequence of a non-antigen-binding fragment resulting from digestion of an antibody or produced by other means, whether in monomeric or multimeric form, and can contain the hinge region. Fc molecules are made up of monomeric polypeptides that can be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of typical Fc molecules ranges from 1 to 4 depending on class (e.g., IgG, IgA, and IgE) or subclass (e.g., IgG1, IgG2, IgG3, IgA1, IgGA2, and IgG4). One example of a Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG.

[0029] The antibodies described herein may be isolated. The term "isolated protein", "isolated polypeptide" or "isolated antibody" refers to a protein, polypeptide or antibody that by virtue of its origin or source of derivation (1) is not associated with naturally associated components that accompany it in its native state, (2) is substantially free of other proteins from the same species, (3) is expressed by a cell from a different species, and/or (4) does not occur in nature. Thus, a polypeptide that is chemically synthesized or synthesized in a cellular system different from the cell from which it naturally originates will be "isolated" from its naturally associated components. A protein may also be rendered substantially free of naturally associated components by isolation, using protein purification techniques well known in the art.

[0030] In some embodiments, the antibodies provided herein comprise HC and LC amino acid sequences as provided in Table 1.

Table 1

mAb	HC SEQ ID NO:	LC SEQ ID NO:
2	2	7
3	3	7
4	4	7
5	5	8
6	5	9
7	6	8
8	6	9
9	10	7

BINDING PROPERTIES

[0031] The term "affinity" refers to a measure of the attraction between two polypeptides, such as receptor/ligand or antigen/antibody, for example. The intrinsic attractiveness between two polypeptides can be expressed as the binding affinity equilibrium constant (KD) of a particular interaction. An antibody is said to specifically bind to an antigen when the KD is ≤ 1 µM, preferably ≤ 100 nM. A KD binding affinity constant can be measured, e.g., by surface plasmon resonance (SPR) (BIAcore®) or Bio-Layer Interferometry.

[0032] The term "koff" refers to the dissociation rate constant of a particular antibody-antigen interaction. A koff dissociation rate constant can be measured, e.g., by Bio-Layer Interferometry.

BINDING TO CD38

[0033] In some embodiments, the antibodies provided herein comprise a tetrameric protein comprising two HCs and two LCs and binds to human CD38 with an affinity or KD of about 1.91 x 10^{-10} M to about 6.7 x 10^{-10} M when measured by Surface Plasmon Resonance (SPR) as described below. In some embodiments, the HC and LC have the amino acid sequences of SEQ ID NOs: 2 and 7 (mAb 2), 3 and 7 (mAb 3), 4 and 7 (mAb 4), 5 and 8 (mAb 5), 5 and 9 (mAb 6), 6 and 8 (mAb 7), or 6 and 9 (mAb 8), respectively.

[0034] In some embodiments, the antibody comprises HC and LC having the amino acid sequences of SEQ ID NOs: 3 and 7, respectively, and the antibody binds to human CD38 with an affinity or KD of about 2 x 10^{-10} M. In some embodiments, the antibody comprises HC and LC having the amino acid sequences of SEQ ID NOs: 5 and 8, respectively, and the antibody binds to human CD38 with an affinity or KD of about 6.7 x 10^{-10} M. In some embodiments, the antibody comprises HC and LC having the amino acid sequences of SEQ ID NOs: 5 and 9, respectively, and the antibody binds to human CD38 with an affinity or KD of about 4.15 x 10^{-10} M. In some embodiments, the antibody comprises HC and LC having the amino acid sequences of SEQ ID NOs: 6 and 8, respectively, and the antibody binds to human CD38 with an affinity or KD of about 3.85 x 10^{-10} M. In some embodiments, the antibody comprises HC and LC having the amino acid sequences of SEQ ID NOs: 6 and 9, respectively, and the antibody binds to human CD38 with an affinity or KD of about 1.91 x 10^{-10} M.

BINDING TO FcγRIIIa (CD16a) AND FcγRIIa (CD32a)

[0035] The antibodies provided herein are also capable of binding to the lower affinity FcγRIIIa (CD16a) variant having phenylalanine (F) at amino acid position 158 (158F) and are capable of binding to the higher affinity FcγRIIIa (CD16a) variant having valine (V) at amino acid position 158 (158V) with KDs within less than an order of magnitude, respectively, and with KDs of less than 100 nM as measured by SPR. In some embodiments, the antibodies provided herein bind to the 158F variant and to the 158V variant of FcγRIIIa (CD16a) with KDs of less than 100 nM as measured by SPR and where in the binding to the 158F and 158V variants differ by less than 2-fold. In some embodiments, the antibodies provided herein bind to FcγRIIIa (CD16a) variant (158F) with a KD of about 59 nM or less when measured, for example, by SPR.

[0036] In some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 2 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIIa (158F) with a KD of about 53 nM and of binding to FcγRIIIa (158V) with a KD or about 47 nM as measured, for example, by SPR. In some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 3 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIIa (158F) with a KD of about 59 nM and is capable of binding to FcγRIIIa (158V) with a KD of about 75 nM as measured, for example, by SPR. In some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 4 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIIa (158F) with a KD of about 51 nM and is capable of binding to FcγRIIIa (158V) with a KD of about 47 nM as measured, for example, by SPR.

[0037] The antibodies provided herein are also capable of binding to the lower affinity FcγRIIa (CD32a) variant having arginine at amino acid position 131 (131R) with a KD of ≤ 690 nM and are capable of binding to the higher affinity FcγRIIa (CD32a) variant having a histidine at position 131 (131H) with a KD of less than about 270 nM as measured, for example, by SPR.

[0038] In some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 2 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIa (CD32a) (131R) with a KD of about 690 nM and of binding to FcγRIIa (CD32a) (131H) with a KD or about 120 nM as measured, for example, by SPR. n some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 3 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIa (CD32a) (131R) with a KD of ≤ about 125 nM or ≤100 nM and is capable of binding to FcγRIIa (CD32a) (131H) with a KD of about 220 nm as measured, for example, by SPR. In some embodiments, the antibody comprises an HC having an amino acid sequence of SEQ ID NO: 4 and an LC having an amino acid sequence of SEQ ID NO: 7 and is capable of binding to FcγRIIa (CD32a) (131R) with a KD of about 510 nM and is capable of binding to FcγRIIa (CD32a) (131H) with a KD of about 60 nM as measured, for example, by SPR.

[0039] In some embodiments, the antibody is also capable of binding to FcγRIIIa (CD16a) 158F with apparent KD of about 70 nM or less and to FcγRIIIa (CD16a) 158V with apparent KD of about 32 nM or less as measured by binding to FcγRIIIa (CD16a) 158F- or 158V-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 2 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIIa (CD16a) 158F with apparent KD of about 70 nM and is capable of binding FcγRIIIa (CD16a) 158V with apparent KD of about 18 nM as measured by binding to FcγRIIIa (CD16a) 158F- or 158V-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 3 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIIa (CD16a) 158F with apparent KD of about 60 nM and is capable of binding FcγRIIIa (CD16a) 158V with apparent KD of about 32 nM as measured by binding to FcγRIIIa (CD16a) 158F- or 158V-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 4 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIIa (CD16a) 158F with apparent KD of about 44 nM and is capable of binding FcγRIIIa (CD16a) 158V with apparent KD of about 28 nM as measured by binding to FcγRIIIa (CD16a) 158F- or 158V-expressing HEK cells, respectively.

[0040] In some embodiments, the antibody is also capable of binding to FcγRIIa (CD32a) variant having arginine at amino acid position 131 (131R) with apparent KD of about 890 nM or less and to FcγRIIa (CD32a) variant 131H with apparent KD of about 840 nM or less as measured by binding to FcγRIIa (CD32a) 131R- or 131H-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 2 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIa (CD32a) 131R with apparent KD of about 890 nM and is capable of binding FcγRIIa (CD32a) 131H with apparent KD of about 840 nM as measured by binding to FcγRIIa (CD32a) 131R- or 131H-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 3 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIa (CD32a) 131R with apparent KD of about 87 nM and is capable of binding FcγRIIa (CD32a) 131H with apparent KD of about 222 nM as measured by binding to FcγRIIa (CD32a) 131R- or 131H-expressing HEK cells, respectively. In some embodiments, the antibody comprising an HC having an amino acid sequence of SEQ ID NO: 4 and an LC having an amino acid sequence of SEQ ID NO: 7 is also capable of binding to FcγRIIa (CD32a) 131R with apparent KD of about 467 nM and is capable of binding FcγRIIa (CD32a) 131H with apparent KD of about 544 nM as measured by binding to FcγRIIa (CD32a) 131R- or 131H-expressing HEK cells, respectively.

MECHANISM OF ACTION

[0041] The antibodies provided herein are capable of killing a CD38-expressing cell by Antibody Dependent Cellular Cytotoxicity (ADCC) of cells expressing high (~400,000/cell), medium (~100,000/cell), and low (~13,000/cell) levels of CD38 molecules on the cell surface. The antibodies provided herein trigger such ADCC in the presence of Natural Killer (NK) cells that express the lower affinity FcγIIIa (CD16a) receptor variant (158F) and/or express the higher affinity FcγIIIa (CD16a) receptor variant (158V). ADCC can be measured using methods known in the art, for example by measuring target cell lysis in the presence of the antibody and effector cells in a cell-based potency assay. The target cell can be, for example, a CD38 expressing cell such as KMS12-BM, RPMI-8226, or MOLP-8. In addition, the effector cells can be, for example, any cell that can be induced to kill target cells by ADCC. In some embodiments the effector cells are primary cells isolated from human blood such as peripheral blood mononuclear cells (PBMC) or human NK cells purified from PBMC. In another embodiment, the cells are a Natural Killer cell line such as NK-92 cells that express FcγRIIIa (158V) or FcγRIIIa (158F) on the surface of the cells (see WO 06/023148). In still other embodiments, the NK cell line is a cell line such as a Jurkat cell line that has been engineered to express FcγIIIa (CD16a) 158F or 158V (see for example Promega, G701A).

[0042] The antibodies provided herein are capable of killing cells expressing a high level of CD38 on the cell surface by Antibody Dependent Cellular Phagocytosis (ADCP). The antibodies provided herein trigger such ADCP in the presence of human PBMC. In one embodiment, the antibodies provided herein trigger about 41% phagocytosis of CD38-expressing cells by human PMBCs that express FcγRIIIa (158V) with a relative EC50 of 212.6 pM.

PHARMACEUTICAL COMPOSITIONS AND FORMULATED ANTIBODIES

[0043] The term "pharmaceutical formulation" refers to a preparation which is in such form as to permit the biological activity of the active ingredient to be effective, and which contains no additional components which are unacceptably toxic to a subject to which the formulation would be administered. Such formulations typically are sterile. "Pharmaceutically acceptable" excipients (vehicles, additives) are those which can reasonably be administered to a subject mammal to provide an effective dose of the active ingredient employed.

[0044] Pharmaceutical compositions and formulations of the antibodies provided herein can be prepared by mixing the antibody, having the desired degree of purity, with one or more optional pharmaceutically acceptable carriers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), and can be provided in the form of lyophilized formulations or aqueous solutions.

[0045] In some embodiments, the pharmaceutical compositions provided herein comprise a formulated antibody comprising an antibody and one or more of the following excipients: sucrose, L-histidine, and polysorbate 80 (PS80). In some embodiments, the antibodies comprise HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1. In some embodiments, the antibody is present in the pharmaceutical composition at a concentration of 5 mg/mL to 50 mg/mL. In some embodiments, the antibody is present in the pharmaceutical composition at a concentration of 5 mg/mL. In some embodiments, the antibody is present in the pharmaceutical composition at a concentration of 10 mg/mL. In some embodiments, the antibody is present in the pharmaceutical composition at a concentration of 20 mg/mL. In some embodiments, the antibody is present in the pharmaceutical composition at a concentration of 50 mg/mL. The concentration of each excipient is chosen such that the pharmaceutical composition can be diluted for administration by infusion. For example, in some embodiments, the sucrose is present at a concentration of 8 % (w/v) to 10 % (w/v). In some embodiments, the sucrose is present at a concentration of 8 % (w/v). In other embodiments, the sucrose is present at a concentration of 10% (w/v). In some embodiments, the L-histidine is present at a concentration of 10 mM to 20 mM. In some embodiments, the L-histidine is present at a concentration of 10 mM. In some embodiments, the L-histidine is present at a concentration of 20 mM. In some embodiments, the PS80 is present at a concentration of 0.005 % (v/v) to 0.05 % (v/v). In some embodiments, the PS80 is present at a concentration of 0.005 % (v/v). In some embodiments, the PS80 is present at a concentration of 0.02 % (v/v). In some embodiments, the PS80 is present at a concentration of 0.05 % (v/v). In some embodiments, the pH of the pharmaceutical composition is optimized to maintain stability of the antibody for example when the pharmaceutical composition is in liquid form. In some embodiments, the pH of the formulated antibody has a pH of about 6.0 to about 6.5. In some embodiments, the formulated antibody has a pH of about 6.0. In some embodiments, the formulated antibody has a pH of about 6.2. In some embodiments, the formulated antibody has a pH of about 6.5. In some embodiments, the formulated antibody has a pH of 6.0. In some embodiments, the formulated antibody has a pH of 6.2. In some embodiments, the formulated antibody has a pH of 6.5.

[0046] In some embodiments, the antibody of the pharmaceutical composition and/or formulated antibodies provided herein comprise one or more charged isoforms. Charged isoforms are typically described as main isoform, acidic isoform(s), and basic isoform(s). The main isoform refers to the most prevalent isoform in a given batch of antibody. Acidic isoform(s) have lower isoelectric point (pI) compared to the main isoform, and basic isoforms have higher pH compared to the main isoform. Charged isoforms can result from post-translational modifications to the amino acid sequence of the HC and/or LC. For example, increases in deamidation, glycation and sialylation, may cause a decrease in the pI of an antibody. Conversion of an N-terminal glutamic acid to pyroGlu (or pyroQ) results in the loss of one positive charge, which may cause a decrease in the pI. In addition, the presence of a C-terminal lysine may cause an increase the pI of an antibody. Furthermore, amidation of a C-terminal proline may cause an increase in pI of the antibody.

[0047] In some embodiments of the antibodies (including antibodies in the pharmaceutical composition, and formulated antibodies) provided herein, the amino acid at position 1 of the HC amino acid sequence is pyroglutamine. In some embodiments of the antibodies provided herein, the HC has a C-terminal amino acid consisting of glycine.

[0048] Charged isoforms of the antibodies provided herein can be separated and quantified using methods known in the art for separating polypeptides based on charge. For example, in some embodiments, a weak cation exchange column can be used in a high performance liquid chromatography (HPLC) system with a phosphate/sodium chloride gradient buffer. In this system, following loading of the antibody onto the column, the acidic isoforms of the antibody would elute first, followed by the main isoform and then the basic isoforms of the antibody. In another embodiment, capillary isoelectric focusing (cIEF) can be used. Capillary isoelectric focusing is a method that separates proteins by their isoelectric point (pI) values. In cIEF, the antibody sample migrates to its isoelectric points on a pH gradient within the capillary, thereby resolving the different charge isoforms along the length of the capillary. To identify and characterize the cIEF variants, charge isoforms can be isolated by strong cation exchange chromatography (SCX) and analyzed by cIEF. An image of the resolved charged isoforms in the capillary is taken using a charge coupled device camera using whole column detection and measuring absorbance at 280 nm. The charge isoform distribution of the test sample is compared to a reference standard using cIEF to identify the charge isoforms of the antibody. In some embodiments, cIEF is used as a quality control measure to confirm the identity of the antibody during commercial production, and by determining the charge isoform distribution of the antibody in comparison to a reference standard, e.g., a test antibody having a charge isoform distribution pattern within a predefined distribution pattern or compared to the distribution pattern of a reference standard.

[0049] In some embodiments, the antibodies (including antibodies in the pharmaceutical composition, and formulated antibodies) comprise HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1, and one or more charged isoform parameters selected from the group consisting of: at least about 70% main isoform, no more than about 30% acidic isoform, and less than 4% basic isoform. In some embodiments, the antibody comprises 71% main isoform, 28% acidic isoform, and less than 4% basic isoform. In some embodiments the antibody contains charge isoforms with isoelectric points ranging from about 5.8 to about 9.0. In some embodiments the antibody contains charge isoforms with isoelectric points ranging from about 5.85 to about 8.97.

[0050] In some embodiments, the antibodies (including antibodies in the pharmaceutical composition, and formulated antibodies) are lyophilized. The antibodies (including antibodies in the pharmaceutical composition, and formulated antibodies) can be in a container (e.g., a vial) such that a prescribed dose of the antibody can be removed from the container for administration to a patient.

[0051] Unit dosage forms of formulated antibodies that specifically bind CD38 are provided herein. In some embodiments, the antibodies comprise HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1, and the unit dosage form comprises the antibody and one or more excipients selected from the group of sucrose, L-histidine, and polysorbate 80. In some embodiments, the unit dosage form comprises about 215 mg of the antibody, about 6.21 mg L-histidine, about 344 mg sucrose, and about 2.15 mg polysorbate 80. In some embodiments, the unit dosage form comprises 215 mg of the antibody, 6.21 mg L-histidine, 344 mg sucrose, and 2.15 mg polysorbate 80. In some embodiments, the unit dosage form is lyophilized. The unit dosage form can be in a container (e.g., a vial) such that a prescribed dosage of the antibody can be removed from the container for administration to a patient. In some embodiments, the antibody, pharmaceutical formulation, and/or formulated antibody are in a glass vial fitted with elastomeric closure. In some embodiments, the vial contains about 215 mg of antibody. In some embodiments, the vial contains 215 mg of antibody. In some embodiments, the fill volume of the vial has been established to enable removal of about 4 mL. In some embodiments, the fill volume of the vial has been established to enable removal of 4 mL.

PROCESS FOR PREPARING

[0052] Provided herein are processes for preparing the antibody, pharmaceutical composition, and unit dosage forms. In some embodiments, the antibodies comprise HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1. In one embodiment, the process comprises expressing the antibody in a suitable cell culture. The antibody is subjected to at least one of a chromatography step and at least one ultrafiltration step, thereby producing a purified antibody solution. The purified antibody solution is adjusted to concentrate the antibody and to add excipients and adjust pH. In one embodiment, the concentration of the antibody is adjusted to be about 50 mg/ml; sucrose, L-histidine, and polysorbate 80 are added, and the pH is adjusted to be at least about 6.0. In one embodiment, the concentration of the antibody is adjusted to be 50 mg/ml; sucrose, L-histidine, and polysorbate 80 are added, and the pH is adjusted to be at least 6.0. In some embodiments, the pH is about 6.2. In some embodiments, the pH is 6.2. In some embodiments, the sucrose is at a concentration of about 8 % w/v, the L-histidine is at a concentration of about 10 mM; and the polysorbate 80 is at a concentration of about 0.05 % v/v. In some embodiments, the sucrose is at a concentration of 8 % w/v, the L-histidine is at a concentration of 10 mM; and the polysorbate 80 is at a concentration of 0.05 % v/v. In some embodiments, the antibody, pharmaceutical composition and/or formulated antibody are lyophilized.

[0053] In some embodiments methods of preparing reconstituted formulated antibody are provided wherein the formulated antibody is provided in lyophilized form and comprises HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1, sucrose at a concentration of about 8 % w/v, L-histidine at a concentration of about 10 mM, and polysorbate 80 at a concentration of about 0.05 % v/v. In some embodiments methods of preparing reconstituted formulated antibody are provided wherein the formulated antibody is provided in lyophilized form and comprises HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1, sucrose at a concentration of 8 % w/v, L-histidine at a concentration of 10 mM, and polysorbate 80 at a concentration of 0.05 % v/v. In some embodiments, prior to use the formulated antibody is reconstituted in a suitable volume of water for injection to produce a solution comprising the antibody at a concentration of about 50 mg/mL, sucrose at a concentration of about 8 % w/v, L-Histidine at a concentration of about 10 mM, and polysorbate 80 at a concentration of about 0.05 % v/v, wherein the pH of the reconstituted antibody is about 6.2. In some embodiments, prior to use the formulated antibody is reconstituted in a suitable volume of water for injection to produce a solution comprising the antibody at a concentration of 50 mg/mL, sucrose at a concentration of 8 % w/v, L-Histidine at a concentration of 10 mM, and polysorbate 80 at a concentration of 0.05 % v/v, wherein the pH of the reconstituted antibody is 6.2.

[0054] As used herein, the term "treatment" or "treating" refers to clinical intervention designed to alter the natural course of the individual or cell being treated during the course of clinical pathology. Desirable effects of treatment include decreasing the rate of disease progression, ameliorating or palliating the disease state, and remission or improved prognosis. For example, an individual is successfully "treated" if one or more symptoms associated with cancer are mitigated or eliminated, including, but not limited to, reducing the proliferation of cancerous cells, destroying cancerous cells, decreasing symptoms resulting from the disease, increasing the quality of life of those suffering from the disease, decreasing the dose of other medications required to treat the disease, and/or prolonging survival of individuals.

[0055] Provided herein are methods for treating or delaying progression of multiple myeloma (such as relapsed multiple myeloma or relapsed and refractory multiple myeloma) in an individual, comprising administering to a subject in need thereof an effective amount of an anti-CD38 antibody provided herein. In some embodiments, the antibodies comprise HC and LC amino acid sequences of any one of the monoclonal antibodies provided in Table 1. In some embodiments, the antibody is capable of improving survival of mice in a murine MM model, wherein mice in the model express the low affinity variant of human CD16a (158F), and wherein the mice have been injected with 500,000 EL4 cells expressing human CD38. In some embodiments, one or more doses of an anti-CD38 antibody is provided as a lyophilized formulation, wherein prior to administration, the lyophilized formulation is reconstituted forming a reconstituted antibody formulation such that the reconstituted antibody formulation has a pH of 6.2 and comprises the antibody at a concentration of about 50 mg/mL in a liquid comprising about 10 mM L-histidine, about 8 % w/v sucrose, and about 0.05 % v/v polysorbate 80. In some embodiments, one or more doses of an anti-CD38 antibody is provided as a lyophilized formulation, wherein prior to administration, the lyophilized formulation is reconstituted forming a reconstituted antibody formulation such that the reconstituted antibody formulation has a pH of 6.2 and comprises the antibody at a concentration of 50 mg/mL in a liquid comprising 10 mM L-histidine, 8 % w/v sucrose, and 0.05 % v/v polysorbate 80.

[0056] In some embodiments one or more doses of antibody is administered to the patent. In some embodiments, the dose can be about 0.1, about 0.2, about 0.3, about 0.5, about 1.0, about 2.5, about 5, about 10, or about 20 mg/kg of the antibody. In some embodiments, the dose can be 0.1, 0.2, 0.3, 0.5, 1.0, 2.5, 5, 10, and 20 mg/kg of the antibody. In some embodiments the antibody is administered intravenously. In some embodiments, the antibody is administered subcutaneously.

Definitions

[0057] As used in this specification and the appended claims, the singular forms “a”, “an” and “the” include plural referents unless the content clearly dictates otherwise. Thus, for example, reference to “a molecule” optionally includes a combination of two or more such molecules, and the like.

[0058] The term “about” as used herein refers to the usual error range for the respective value readily known to the skilled person in this technical field. Reference to “about” a value or parameter herein includes (and describes) embodiments that are directed to that value or parameter *per se*. In some embodiments, the term “about” signifies a stated value plus or minus 10% of said value; for example, “about 10 mg/kg” can encompass 9 to 11 mg/kg. In some embodiments, the term “about” signifies a stated value plus or minus 5% of said value; for example, “about 20 mg/kg” can encompass 19 to 21 mg/kg.

[0059] A “subject” or an “individual” for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal is human.

[0060] Examples have been set forth below for the purpose of illustration and to describe certain specific embodiments of the invention. However, the scope of the claims is not to be in any way limited by the examples set forth herein.

EXAMPLES

1. Antibody Design:

[0061] Fc-engineered anti-CD38 antibodies were generated having mutations in the Fc part of the antibody to enhance the affinity for the activating FcγRIIIa and FcγRIIa receptors on effector cells.

[0062] Two humanized heavy chains (HC) (SEQ ID NO: 5 and SEQ ID NO: 6) carry 14 and 10 mutations in the framework regions (compared to SEQ ID NO: 1) which result in an increase in *Homo sapiens* germinality score from 74.49 % to 88.78 % and 84.69 %, respectively. Two humanized light chains (LC) (SEQ ID NO: 8 and SEQ ID NO: 9) carry 17 and 15 mutations in the framework regions (compared to SEQ ID NO: 7) which result in an increase in *Homo sapiens* germinality score from 64.36 % to 81.19 % and 79.21 %, respectively. In addition, in LC 2 and 3, methionine at position 11 was substituted with leucine to avoid a potential problematic methionine oxidation. The HC and LC sequences are provided in Table 2, and the mAb HC and LC pairings are provided in Table 3.

Table 2

SEQ ID NO:	CHAIN	AMINO ACID SEQUENCE
1	HEAVY CHAIN 1	QVQLVQSGAE VAKPGTSVKL SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGKATL TADKSSKTVY MHLSSLASED SAVYYCARGD YYGSNSLDYW GQGTSVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPGK
2	HEAVY CHAIN 2	QVQLVQSGAE VAKPGTSVKL SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGKATL TADKSSKTVY MHLSSLASED SAVYYCARGD YYGSNSLDYW GQGTSVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PDVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPEEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

SEQ ID NO:	CHAIN	AMINO ACID SEQUENCE
3	HEAVY CHAIN 3	QVQLVQSGAE VAKPGTSVKL SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGKATL TADKSSKTVY MHLSSLASED SAVYYCARGD YYGSNSLDYW GQGTSVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLAG PDVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPEEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
4	HEAVY CHAIN 4	QVQLVQSGAE VAKPGTSVKL SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGKATL TADKSSKTVY MHLSSLASED SAVYYCARGD YYGSNSLDYW GQGTSVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLAG PDVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPLPEEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
5	HEAVY CHAIN 5	QVQLVQSGAE VAKPGASVKV SCKASGYTFT DYWMQWVRQA PGQGLEWIGT IYPGDGDTSY AQKFQGRVTM TADTSTSTVY MELSSLRSED TAVYYCARGD YYGSNSLDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLAG PDVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPEEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
6	HEAVY CHAIN 6	QVQLVQSGAE VAKPGASVKV SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGRVTM TADKSTSTVY MELSSLRSED TAVYYCARGD YYGSNSLDYW GQGTLVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLAG PDVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPEEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG

SEQ ID NO:	CHAIN	AMINO ACID SEQUENCE
7	LIGHT CHAIN 1	DIVMTQSHLS MSTSLGDPVS ITCKASQDVS TVVAWYQQKP GQSPRRLIYS ASYRYIGVPD RFTGSGAGTD FTFTISSVQA EDLAVYYCQQ HYSPPYTFGG GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
8	LIGHT CHAIN 2	DIVMTQSPDS LAVSLGERAT INCKSSQDVS TVLAWYQQKP GQSPRRLIYS ASYRYIGVPD RFSGSGSGTD FTLTISSLQA EDVAVYYCQQ HYSPPYTFGG GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
9	LIGHT CHAIN 3	DIVMTQSPDS LAVSLGERAT INCKSSQDVS TVVAWYQQKP GQSPRRLIYS ASYRYIGVPD RFSGSGSGTD FTFTISSLQA EDVAVYYCQQ HYSPPYTFGG GTKLEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC
10	HEAVY CHAIN 7	QVQLVQSGAE VAKPGTSVKL SCKASGYTFT DYWMQWVKQR PGQGLEWIGT IYPGDGDTGY AQKFQGKATL TADKSSKTVY MHLSSLASED SAVYYCARGD YYGSNSLDYW GQGTSVTVSS ASTKGPSVFP LAPSSKSTSG GTAALGCLVK DYFPEPVTVS WNSGALTSGV HTFPAVLQSS GLYSLSSVVT VPSSSLGTQT YICNVNHKPS NTKVDKKVEP KSCDKTHTCP PCPAPELLGG PSVFLFPPKP KDTLMISRTP EVTCVVVDVS HEDPEVKFNW YVDGVEVHNA KTKPREEQYN STYRVVSVLT VLHQDWLNGK EYKCKVSNKA LPAPIEKTIS KAKGQPREPQ VYTLPPSRDE LTKNQVSLTC LVKGFYPSDI AVEWESNGQP ENNYKTTPPV LDSDGSFFLY SKLTVDKSRW QQGNVFSCSV MHEALHNHYT QKSLSLSPG
11	huCD16a (FcγRIIIa) UniProtKB/ Swiss-Prot P08637 “158V” variant bold	MWQLLLPTAL LLLVSAGMRT EDLPKAVVFL EPQWYRVLEK DSVTLKCQGA YSPEDNSTQW FHNESLISSQ ASSYFIDAAT VDDSGEYRCQ TNLSTLSDPV QLEVHIGWLL LQAPRWVFKE EDPIHLRCHS WKNTALHKVT YLQNGKGRKY FHHNSDFYIP KATLKDSGSY FCRGL**V**GSKN VSSETVNITI TQGLAVSTIS SFFPPGYQVS FCLVMVLLFA VDTGLYFSVK TNIRSSTRDW KDHKFKWRKD PQDK

SEQ ID NO:	CHAIN	AMINO ACID SEQUENCE
12	huFcγRIIa UniProtKB/ Swiss-Prot P12318-1 "131H" variant bold	MTMETQMSQN VCPRNLWLLQ PLTVLLLLAS ADSQAAAPPK AVLKLEPPWI NVLQEDSVTL TCQGARSPES DSIQWFHNGN LIPTHTQPSY RFKANNNDSG EYTCQT**G**QTS LSDPVHLTVL SEWLVLQTPH LEFQEGETIM LRCHSWKDKP LVKVTFFQNG KSQKFSHLDP TFSIPQANHS HSGDYHCTGN IGYTLFSSKP VTITVQVPSM GSSSPMGIIV AVVIATAVAA IVAAVVALIY CRKKRISANS TDPVKAAQFE PPGRQMIAIR KRQLEETNND YETADGGYMT LNPRAPTDDD KNIYLTLPPN DHVNSNN
13	HEAVY CHAIN 8	EVQLLESGGG LVQPGGSLRL SCAVSGFTFN SFAMSWVRQA PGKGLEWVSA ISGSGGGTYY ADSVKGRFTI SRDNSKNTLY LQMNSLRAED TAVYFCAKDK ILWFGEPVFD YWGQGTLVTV SSASTKGPSV FPLAPSSKST SGGTAALGCL VKDYFPEPVT VSWNSGALTS GVHTFPAVLQ SSGLYSLSSV VTVPSSSLGT QTYICNVNHK PSNTKVDKRV EPKSCDKTHT CPPCPAPELL GGPSVFLFPP KPKDTLMISR TPEVTCVVVD VSHEDPEVKF NWYVDGVEVH NAKTKPREEQ YNSTYRVVSV LTVLHQDWLN GKEYKCKVSN KALPAPIEKT ISKAKGQPRE PQVYTLPPSR EEMTKNQVSL TCLVKGFYPS DIAVEWESNG QPENNYKTTP PVLDSDGSFF LYSKLTVDKS RWQQGNVFSC SVMHEALHNH YTQKSLSLSP GK
14	LIGHT CHAIN 4	EIVLTQSPAT LSLSPGERAT LSCRASQSVS SYLAWYQQKP GQAPRLLIYD ASNRATGIPA RFSGSGSGTD FTLTISSLEP EDFAVYYCQQ RSNWPPTFGQ GTKVEIKRTV AAPSVFIFPP SDEQLKSGTA SVVCLLNNFY PREAKVQWKV DNALQSGNSQ ESVTEQDSKD STYSLSSTLT LSKADYEKHK VYACEVTHQG LSSPVTKSFN RGEC

Table 3

mAb	Heavy Chain SEQ ID NO:	Light Chain SEQ ID NO:
1	1	7
2	2	7
3	3	7
4	4	7
5	5	8
6	5	9
7	6	8
8	6	9

9	10	7
10	13	14

2. ***Biochemical Characterization:***

A: Binding to CD38

[0063] In one experiment, binding affinity and kinetic constants of mAbs 1-8 for human CD38 was measured by SPR using a Biacore® instrument. Briefly, anti-CD38 antibodies were captured to a CM5 chip to which anti-Fc antibody had been covalently bound. Multiple concentrations of huCD38 were injected over the captured antibodies being tested. Binding curves (sensorgrams) were generated and kinetic analyses were performed using the Biacore Evaluation software.

[0064] As shown in Table 4, mAbs 3 and 8 had the highest affinity for human CD38 (T-tests for each variant: p-value >2).

Table 4. Affinity for human CD38 (n=3)

mAb	ka (1/Ms)	CV ka (%)	kd (1/s)	CV kd (%)	KD (M)	CV Kd (%)
1	3.55E+06	11.6	6.55E-04	3.4	1.80E-10	5.0
3	3.89E+06	13.8	6.59E-04	2.2	1.71E-10	13.1
5	2.78E+06	11.0	1.88E-03	29.1	6.99E-10	22.1
6	3.10E+06	15.8	8.55E-03	76.2	3.85E-10	18.7
7	3.18E+06	15.4	1.30E-03	9.5	4.15E-10	14.5
8	3.51E+06	19.2	6.50E-04	4.7	1.91E-10	24.0

[0065] In another experiment, binding affinity of mAbs 1 and 3 to human CD38 was measured by SPR using a Biacore® instrument as described above. mAb 1 has a Kd of 0.22 nM and mAb 3 had a Kd of 0.20 nM.

[0066] Effect of thermal stress on the binding affinity to human CD38: Binding affinity and kinetic constants of mAbs 1-8 for human CD38 after subjecting the mAbs to thermal stress (14 days at 40° C in 10 mM histidine pH6, 8% sucrose, 0.02% PS80) was measured by SPR using a Biacore® instrument. Same protocol described in the paragraph above has been used.

[0067] As shown in Table 5, the thermally stressed samples showed very similar affinity and kinetic constants as the corresponding non-stressed samples CD38 (Table 4) (T-tests for each variant: p-value >2).

Table 5. Effect of thermal stress on affinity for human CD38 (n=3)

mAb	ka (1/Ms)	CV ka (%)	kd (1/s)	CV kd (%)	KD (M)	CV KD (%)
1	4.00E+06	21.9	7.14E-04	5.5	1.83E-10	15.7
3	3.71E+06	8.2	6.71E-04	3.0	1.81E-10	5.3
5	2.95E+06	21.2	2.36E-03	4.4	8.28E-10	25.7
6	3.86E+06	27.9	1.28E-03	3.7	3.46E-10	24.0
7	3.39E+06	6.8	1.33E-03	5.9	3.94E-10	10.5
8	3.53E+06	25.2	6.63E-04	11.7	1.98E-10	31.5

B: Measurement of affinity for FcγRIIa, FcγRIIIa, and FcγRIIb **in silico**

[0068] In one experiment, affinity of the mAbs for FcγRIIIa 158V receptors or FcγRIIIa 158F receptors was measured using Surface Plasmon Resonance (SPR). The mAbs were produced in HEK293 cells by transient transfection according to supplier (ThermoFisher Scientific), and purified by protein A affinity chromatography according to supplier (GE Healthcare). FcγRIIIa (CD16a) extracellular domains with V158 or F158 were produced with a C-terminal histag in HEK293 cells by transient expression and purified by Immobilized Metal Affinity Chromatography (IMAC) according to the supplier (Ni-Sepharose GE, Healthcare). Affinity by SPR on BIACore 2000 (GE Healthcare) was performed by capture assay using immobilized anti-His IgG on CM5 chip. FcγRIIIa-histag was then added to the running buffer, followed afterwards by the anti-CD38 mAb at concentrations ranging from 8 to 256 nM. Analysis was performed using the two-state reaction with acceptable χ^2 values of less than 5 (meaning 2% of the highest tested concentration) and a percentage of maximal theoretical resonance unit greater than 25 (meaning more than ¼ productive interaction), using BIAevaluation Software 4_1 (GE Healthcare).

[0069] As shown in Table 6, mAbs 2-4 have a 9-fold higher affinity for the FcγRIIIa 158V than mAb 1 and more than 27-fold higher affinity for the FcγRIIIa 158F receptor than mAb 1.

Table 6

	K_D (nM)	
mAb	FcγRIIIa 158V	FcγRIIIa 158F
1	425	>1500
2	47	53
3	50	55
4	47	51

[0070] In another experiment, the affinity of mAbs 1 and 3 for FcγRIIIa 158V receptors or FcγRIIIa 158F receptors was measured using SPR as described above. mAb 3 bound FcγRIIIa 158V with a KD of 75 nM, and mAb 3 bound FcγRIIIa 158F with a KD of 59 nM.

[0071] The affinity of the mAbs for FcγRIIa receptors having arginine at amino acid position 131 (FcγRIIa 131R) or histidine at amino acid position 131 (FcγRIIa 131H) was measured using AlphaScreen proximity assay.

[0072] FcγRIIa extracellular domains with R131 or H131 were provided by R&D System (batch 1330-CD) or Biorbyt (batch ORB138408), respectively. The assay was a bead based proximity assay described by supplier (Perkin Elmer), permitting a ranking of the anti-CD38 Fc variants by competition to anti-CD38 with native IgG1. Biotinylated-anti-CD38 with native IgG1 was captured on the donor beads, and FcγRIIa histag-protein was captured on the acceptor beads. When anti-CD38 and FcγRIIa interact, beads are brought into proximity. Excitation of the donor beads at 680 nm causes the release of singlet oxygen, which diffuses and triggers the emission of light at 520-620 nm from the acceptor beads, when in proximity. The amount of light is directly proportional to the degree of interaction and it is measured with the EnVision multimode plate reader (Perkin Elmer). During the competition assay, the untagged competitive mAbs anti-CD38 Fc-variants DE (S239D/I332E, Eu numbering), ADE (G236A/S239D/I332E, Eu numbering), or ADLE (G236A/S239D/F243L/I332E, Eu numbering) were used to compete the interaction between the biotinylated-anti-CD38 with native IgG1 and FcγRIIa-histag protein. The concentration of biotinylated-anti-CD38 was set from a calibration curve performed in advance. The untagged competitive mAbs were used at varying levels such that increasing concentration of untagged mAbs can displace the biotinylated anti-CD38 and decreases the signal. Data were normalized for maximal signal. Two experiments were run and IC50 evaluated by BIOST@T-SPEED-LTS 2.1.0.

[0073] As shown in Table 7, mAb 3 showed greater than 14- and 18-fold higher affinity for FcγRIIa 131R and FcγRIIa 131H, respectively, compared to mAb 1.

Table 7. Affinity to FcγRIIa R131 and FcγRIIa H131

	Relative IC50 (nM)	
mAb	FcγRIIa R131	FcγRIIa H131
1	1741	268
2	687	119
3	125	15
4	508	58

C: Measurement of affinity for FcγRIIa, FcγRIIIa, and FcγRIIb **in vitro**

[0074] HEK293T-FcγRIIIa-158F, HEK293T-FcγRIIIa-158V, HEK293T-FcγRIIa-131H, HEK293T-FcγRIIa-131R, HEK293T-FcγRI or HEK293T-FcγRIIb were seeded in 96-well microplates at 10^5 cells/well. The plates were centrifuged at 800 g for 1 minute and resuspended in 50 µL PBS containing the antibodies at different concentrations for 30 minutes at 4°C. 200 µL PBS 1% FBS was then added to wash the cells, followed by a 800 g centrifugation for 1 minute. The washing step was repeated three times in total before staining the cells with the secondary antibody conjugated with FITC (Fragment Goat Anti-human IgG (H+L) FITC, ref 109-546-088 Jackson ImmunoResearch, 10 µg/mL final) for 30 minutes at 4°C. Cells were washed with 200 µL PBS 1% FBS three times and resuspended in 100 µL PBS before acquisition on MACSVYB (B1 channel). As shown in Table 8, mAb 2, 3 and 4 had a 32-, 38-, and 51-fold higher affinity for FcγRIIIa 158F expressed on HEK cells compared to mAb 1, respectively, and mAb 2, 3 and 4 had a 9-, 5-, and 6-fold higher affinity for FcγRIIIa 158V expressed on HEK cells compared to mAb 1, respectively.

Table 8

	mAb	Mean apparent Kd (nM)	SEM	Fold difference in affinity versus mAb 1
FcγRIIIa 158F	1	2269.1	771.4	1
	2	70.1	13.1	32.4
	3	60.4	10.4	37.6
	4	44.2	9.1	51.4
FcγRIIIa 158V	1	165.3	44.2	1
	2	17.6	3.3	9.4
	3	32.0	5.3	5.2
	4	28.1	7.8	5.9

[0075] As shown in Table 9, mAb 3 had a ~5-fold higher affinity for FcγRIIa 131H expressed on HEK cells compared to mAb 1, and mAb 3 had a ~16-fold higher affinity for FcγRIIa 131R expressed on HEK cells compared to mAb 1.

Table 9

	mAb	Mean Kd (nM)	SEM	difference in affinity versus mAb 1
FcγRIIa 131H	1	1180.8	406.1	1
	2	839.8	438.7	1.4
	3	221.9	74.9	5.3
	4	543.8	277.7	2.2
FcγRIIa 131R	1	1420.3	166.5	1
	2	891.1	131.1	1.6
	3	87.4	13.9	16.3
	4	467.3	153.6	3.0

[0076] As shown in Table 10, mAb 2, 3 and 4 had a similar level of binding affinity for FcγRI compared to mAb 1 and a 1.8- to 3.9-fold lower affinity for FcγRIIb expressed on HEK cells compared to mAb 1.

Table 10

	mAb	Mean Kd (nM)	SEM	difference in affinity versus mAb 1
FcγRI	1	24.2	4.2	1
	2	13.5	1.7	1.8
	3	14.7	3.2	1.6
	4	15.9	2.7	1.5
FcγRIIb	1	1299.1	406.1	1
	2	839.8	438.7	3.9
	3	221.9	74.9	3.3
	4	543.8	277.7	1.8

***D: Measurement of cytotoxic activities* in vitro**

ANTIBODY DEPENDENT CELLULAR CYTOTOXICITY (ADCC):

[0077] Antibody Dependent Cell Cytotoxic (ADCC) assays using NK92 cell line engineered to over-express the FcγRIIIa receptor as effector cells were examined using a calcein-acetyoxymethyl (Calcein-AM; Invitrogen) release assay. In live cells, the non-fluorescent calcein AM is converted to a green-fluorescent calcein. The labeled target cells are incubated with SAR442085 antibody and the NK-92 effector cells. Lysis of the target cells through ADCC leads to release of the fluorescent calcein. The intensity of fluorescence is proportional to the level of ADCC activity present.

[0078] The multiple myeloma target cells (MOLP-8, RPMI 8226, MM1R or KMS12-BM) were labeled with Calcein-AM (50 µg diluted in 25 µL DMSO, then diluted 10 µL calcein in 4 mL RPMI 1640 + 1% FBS + 1% Probenecid for staining of 4×10^6 cells) for 30 min, then washed and plated onto 96-well round bottom plates at a density of 2×10^4 cells/well. The indicated test mAbs or control isotype mAb were added at various concentrations from 10 μg/mL to 0.01 pg/mL for 30 min to allow opsonization before adding Natural Killer (NK) cells transduced with the indicated FcγRIIIa variant. NK cells expressing 158F or 158V were added as effector cells at an E:T ratio of 5:1 (1×10^5 NK cells for 2×10^4 target cells). The plates were then incubated for 1 h at 37 °C in a humidified incubator with 5% CO_2, and 100 μL of supernatants were harvested and transferred into opaque 96-well microplates for analysis using fluorometry on Tecan Infinite M1000 to measure calcein release (excitation filter: 492 nm; emission: 515 nm). For maximal release, the cells were lysed with 2% Triton X-100. The fluorescence value of the culture medium background was subtracted from that of the experimental release (A), the target cell spontaneous release (B), and the target cell maximal release (C). The cytotoxicity and ADCC percentages for each plate (in duplicate) were calculated using the following formula:

$$\text{Cytotoxicity (\%)} = (A - B)/(C - B) \times 100\%$$

[0079] Each experiment was repeated at least 3 times. The half-maximal effective concentration (EC50) values were calculated by fitting the data points to a 4-parameter equation using GraphPad Prism 5 (GraphPad Software, Inc., San Diego, CA).

High CD38 receptor density target cells

[0080] ADCC of multiple myeloma cell line MOLP-8 was assessed *in vitro* as described above. MOLP-8 cells exhibit a high CD38 receptor density on the cell surface (~400,000/cell).

[0081] Table 11 shows EC50 of each tested antibody against MOLP-8 cells in the presence of NK cells expressing 158V. As shown in Table 11, in the presence of NK cells expressing the high affinity variant of FcγRIIIa (158V), mAbs 2, 3, and 4 triggered ADCC of MOLP-8 cells with an EC50 of 24- to 33-fold lower than mAb 1.

Table 11

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	3.3	0.91
2	0.1	0.03
3	0.14	0.02
4	0.12	0.02

[0082] Table 12 shows EC50 of each tested antibody against MOLP-8 cells in the presence of NK cells expressing 158F. As shown in Table 12, mAb 4 showed the strongest ADCC activity against MOLP-8 cells in the presence of NK cells expressing the low affinity FcγRIIIa variant (158F), with and EC50 of about 97-fold lower than mAb 1. mAbs 2 and 3 triggered ADCC of MOLP-8 cells with an EC50 of about 59- to 69-fold lower than mAb 1 in the presence of NK cells expressing the low affinity FcγRIIIa variant (158F).

Table 12

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	16.5	8.77
2	0.24	0.11
3	0.28	0.09
4	0.17	0.05

Medium CD38 receptor density target cells

[0083] ADCC of multiple myeloma cell line RPMI-8226 was assessed *in vitro* as described above. RPMI-8226 cells exhibit a medium CD38 receptor density on the cell surface (~70,000/cell).

[0084] Table 13 shows EC50 of each tested antibody against RPMI-8226 cells in the presence of NK cells expressing 158V. As shown in Table 13, mAbs 2-4 demonstrated similar levels of enhanced ability with an EC50 of about 27-fold lower compared to mAb 1 to trigger ADCC against MM cells expressing a medium CD38 receptor density in the presence of NK cells expressing the higher affinity variant of FcγRIIIa (158V).

Table 13

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	2.45	0.73
2	0.09	0.02
3	0.09	0.003
4	0.09	0.003

[0085] Table 14 shows EC50 of each tested antibody against RPMI-8226 cells in the presence of NK cells expressing 158F. mAb 4 demonstrated about a 73-fold enhanced ability compared to mAb 1 to trigger ADCC against MM cells expressing a medium CD38 receptor density in the presence of NK cells expressing the low affinity variant of FcγRIIIa, while mAb 3 demonstrated about a 65-fold and mAb 2 demonstrated about a 49-fold enhanced ability to trigger ADCC against MM cells expressing a medium CD38 receptor density in the presence of NK cells expressing the low affinity variant of FcγRIIIa compared to mAb 1.

Table 14

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	5.83	1.67
2	0.12	0.04
3	0.09	0.01
4	0.08	0.01

Low CD38 receptor density target cells

[0086] ADCC of multiple myeloma cell line KMS-12BM was assessed *in vitro* as described above. KMS-12BM cells exhibit a low CD38 receptor density on the cell surface (~13,000/cell).

[0087] Table 15 shows EC50 of each tested antibody against KMS-12BM cells in the presence of NK cells expressing the higher affinity variant of FcγRIIIa (158V). Interestingly, as shown in Table 15, mAbs 2, 3, and 4 demonstrated enhanced ability compared to mAb 1 to trigger ADCC against MM cells expressing a low CD38 receptor density in the presence of NK cells expressing the higher affinity variant of FcγRIIIa (158V). mAb 3 had an EC50 of ~69-fold lower than mAb 1, and mAb 4 had an EC50 of ~91-fold lower than mAb 1.

Table 15

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	41	12
2	0.65	0.05
3	0.6	0.1
4	0.45	0.35

[0088] Table 16 shows EC50 of each tested antibody against KMS-12BM cells in the presence of NK cells expressing the lower affinity variant of FcγRIIIa (158F). Remarkably, as shown in Table 16, mAbs 2, 3, and 4 demonstrated enhanced ability to trigger ADCC compared to mAb 1 against MM cells expressing a low CD38 receptor density in the presence of NK cells expressing the lower affinity variant of FcγRIIIa (158F). mAb 3 had an EC50 of ~145-fold lower than mAb 1, and mAb 4 had an EC50 of ~269-fold lower than mAb 1.

Table 16

mAb	EC50 (ng/ml)	SEM (ng/ml)
1	94	2.55
2	1.05	0.25
3	0.65	0.15
4	0.35	0.15

ANTIBODY DEPENDENT CELLULAR PHAGOCYTOSIS (ADCP):

[0089] Human PBMCs were first isolated from buffy coat of seven healthy donors from Etablissement Français du Sang. All these donors exhibited the same FCGR3A and FCGR2A genotype (FcγRIIIa- 158V/V and FcγRIIa- 131H/H). Blood was first collected in a 50 mL Falcon tube. 15 mL of Ficoll-Plaque™ PLUS 96% was added very gently at the bottom of a Sepmate tube, then blood added slowly in the tube before centrifugation at 1,300 g for 10 minutes. The PBMC ring was then collected and washed with 50 mL PBS and another round of centrifugation at 300 g for 5 minutes. The washing process was repeated two times before PBMC staining with anti-CD14 magnetic beads according to manufacturer's instructions (Miltenyi; ref 130-050-201): basically an incubation time at 4°C for 15 minutes before positive selection through the AutoMACS pro (Posseld selection). Monocytes were collected and then washed with 50 mL PBS. Monocytes were cultured for 5 days in RPMI1640, 10% FBS, 2% human inactivated serum, 50 ng/ml GM-CSF in a T75 flask at 37° C 5% CO_2. After 5 days culture, monocytes were differentiated into macrophages. To collect macrophages, accutase (ThermoFisher, ref: A1110501) was put in flasks for 15 minutes at 37° C to detach cells, then 20 mL RPMI 10% FBS 1% Glutamine was added to stop accutase activity. Collected cells were centrifuged at 350 g for 10 minutes followed by a wash in 20 mL PBS and another round of centrifugation at 300 g for 10 minutes. For two experiments, the macrophages used were from a macrophage batch frozen in liquid nitrogen.

[0090] Macrophages were resuspended in diluent C at $20x10^6$ cells/mL (provided in manufacturer's staining kit # PKH26GL-1KT from Sigma Aldrich). For 1 volume of macrophages, 1 volume of PKH26 (20 µL diluted in 1 mL diluent C) was added to the cells for 5 minutes incubation in the dark. 5 mL FBS were then added to inactivate the staining process for 1 minute, before completing to 50 mL with RPMI1640 10% FBS.CD38-expressing MOLP-8 cells were stained with PKH67 fluorochrome and macrophages derived from purified monocytes were stained with PKH26, making them distinguishable by flow cytometry. Then MOLP-8 cells and macrophages were put in culture in the presence of either mAb 1 or mAb 3 overnight before analysis of the double positive population (macrophages that phagocytosed MOLP-8 cells) by flow cytometry.

[0091] mAb 3 exhibited an enhanced ADCP activity as compared to mAb 1 against MOLP-8 cell line as demonstrated by the higher percentage (%) of total phagocytosis achieved in all experiments and the lower EC50 (relative) value (EC50rel). The geometric mean of the % of total phagocytosis was 40.85% for mAb 3 versus 18.57% for mAb 1 and the geometric mean of EC50rel was 31.87 ng/mL (or 212.57 pM) for mAb 3 versus mAb 1 for which the value was over 64.86 ng/mL (or over 432.62 pM). Based on EC50rel values, mAb 3 appeared to be at least 2.035 times better than mAb 1 for the ADCP activity.

***In vivo* efficacy**

[0092] The *in vivo* efficacy of mAb 3 was evaluated. Humanized FcγR C57BL/6 mice (Smith P, DiLillo DJ, Bournazos S, Li F, Ravetch JV; Proc Natl Acad Sci USA. 2012; 109(16):6181-6) were injected intravenously with 500,000 EL4-huCD38 tumor cells on day 0. Humanized FcγR C57BL/6 mice have had all murine genes encoding FcγRs deleted and human FcγRs, encoded as transgenes, inserted into the mouse genome. Humanized FcγR C57BL/6 mice recapitulate huFcγR expression patterns and expression levels and are functional in a variety of huIgG-mediated models of inflammation, cytotoxicity, and tumor clearance. For the activating FcγRIIIa and FcγRIIa receptors, the human variants inserted in the mouse model were, respectively, huFcγRIIIa 158F and huFcγRIIa 131R (the low affinity variants of the receptors). The profile of human FcγR expression pattern and expression levels were recapitulated in the mouse.

[0093] Starting on day 1 post tumor cell injection, the test or control mAbs were administered intraperitoneally on days 1, 4, 7, and 14. The isotype control mAb was administered at 10 mg/kg. mAbs 1, 3, and 10 were administered at 10 and 1.25 mg/kg. In this survival model, the primary efficacy end points were Median Survival Time (MST), the percent Increased Lifespan (%ILS), and the long term survivors (defined as mice with survival duration superior or equal to 2 times the MST of control group). The survival differences between groups were evaluated with a Cox model.

[0094] As shown in Table 17, the isotype control group had an MST of 39 days. Twenty percent of the isotype control group exhibited long term survival with this model. The group treated with mAb 1 at 10 mg/kg had a MST of more than 82.5 days, an increased lifespan greater than 112%. Fifty percent of the group treated with 10 mg/kg of mAb 1 was long term survivors. Mice treated with 1.25 mg/kg of mAb 1 had an MST greater than 46.5 days, and an increased lifespan greater than 19%. Thirty percent of the group treated with 1.25 mg/kg of mAb 1 was long term survivors.

[0095] The group treated with mAb 10 at 10 mg/kg had a MST of more than 70 days, an increased lifespan of 70%. Fifty percent of the group treated with 10 mg/kg of mAb 10 was long term survivors. Mice treated with 1.25 mg/kg of mAb 10 had an MST greater than 42 days, and an increased lifespan of 8%. Only 10 % of the group treated with 1.25 mg/kg of mAb 10 was long term survivors.

[0096] The group treated with mAb 3 at 10 mg/kg had a MST of more than 90 days, an increased lifespan greater than 131%. Remarkably, 90 % of the group treated with 10 mg/kg of mAb 3 was long term survivors. mAb 3 at 10 mg/kg was statistically significantly more active compared to the isotype control group (p=0.0351). Even more surprising, mice treated with 1.25 mg/kg of mAb 3 also had an MST greater than 90 days, and an increased lifespan greater than 131%. Furthermore, 90% of the group treated with 1.25 mg/kg of mAb 3 was long term survivors. mAb 3 at 1.25 mg/kg was statistically significantly more active as compared to the isotype control group (p=0.0380) and statistically significantly better than mAb 10 at the same dose (p=0.0380).

[0097] Treatment of this tumor model in mice bearing the low affinity huFcγRIIIa (F158/F158) with mAb 1, mAb 3, and mAb 10 at the dose of 10 mg/kg resulted in a statistically significant improvement in lifespan and number of long term survivors. Surprisingly, however, at the dose of 1.25 mg/kg, mAb 3 still exhibited a statistically significant improvement in lifespan and number of long term survivors while mAb 1 and mAb 10 were inactive.

Table 17

Agent	Dosage (mg/kg)	Long term survivors (%)	MST (day)	%ILS
Isotype control	10	20	39	-
mAb 3	10	90	>90	131%
	1.25	90	>90	131%
mAb 1	10	50	82.5	112%
	1.25	30	46.5	19%
mAb 10	10	50	70	79%
	1.25	10	42	8%

E. Stability Studies

Thermostability

[0098] The thermostability of mAbs 1, 3, 5, 6, 7, and 8 was measured by differential scanning calorimetry (DSC) using a Malvern MicroCal VP calorimeter. The samples were tested in a buffer comprising 10 mM histidine HCl at pH 6.0. The prepared samples were loaded into wells of a Wheaton 96-well round bottom plate, along with buffer as reference, in triplicate. Using a temperature ramp rate of 1° C/min and a range of 20-120° C, thermal scans were collected and processed using origin software 2.0.

[0099] As shown in Table 18, mAbs 3, 5, 6, 7, and 8 showed an onset of protein conformation change at about 40° C, at a temperature of about 17 degrees lower than the onset temperature of mAb 1 and 15-20° C lower than is typical for mAbs (FIG. 1). Furthermore, unfolding of the CH2 domain of mAbs 3, 5, 6, 7, and 8 began much earlier than for mAb 1, at about 50° C compared to 70° C.

Table 18. Thermostability (° C)

<table>
<tr><th>mAb</th><th>T_{onset}</th><th>T_m1 (CH2)</th><th>T_m2 (Fab1)</th><th>T_m3 (Fab2)</th><th>T_m4 (CH3)</th></tr>
<tr><td>1</td><td>56.8</td><td colspan="2">70.0</td><td>75.7</td><td>83.1</td></tr>
<tr><td>3</td><td>39.9</td><td>50.4</td><td colspan="2">71.0</td><td>83.3</td></tr>
</table>

5	37.8	49.9	79.4	81.9
6	40.7	49.8	81.7	84.1
7	37.2	50.0	78.1	84.3
8	38.8	50.5	80.2	84.8

Isoelectric point

[00100] The isoelectric point (pI) of each of mAbs 1, 3, 5, 6, 7, and 8 was measured by capillary isoelectric focusing (cIEF). The theoretical pIs were determined from composition using the analysis platform SEDNTERP and assuming the following amino acid pKa values: Arg = 12, Asp = 4.5, Glu = 4.6, His = 6.2, Lys = 10.4, and Tyr = 9.7. All sulfhydryl sidechains in Cys residues were assumed disulfide-bonded with no contributing pKa (Laue TM, Shah BD, Ridgeway TM, and Pelletier SL. Computer-aided interpretation of analytical sedimentation data for protein. In Analytical Ultracentrifugation in Biochemistry and Polymer Science. Harding SE, Rowe AJ, and Horton JC Eds. pp 90-124. Royal Society of Chemistry, 1991).

[00101] Surprisingly, as shown in Table 19, the measured pI was lower than the calculated pI for mAb 3 based on the sequence.

Table 19

mAb	Calculated pI	Measured pI	% “acidic” forms	% “basic” forms
1	7.46	7.47	16.6	19.0
3	7.46	6.95	20.1	44.0
5	6.72	5.67	20.7	23.6
6	6.72	5.66	16.8	21.5
7	7.11	6.01	25.8	48.5
8	7.11	6.22	11.5	25.7

Formulation Development of mAb 3

[0102] Based on the thermostability results, a lyophilized formulation was developed to ensure adequate stability of mAb 3 that was suitable for storage of the mAb at ≤ -30° C and for storage of the lyophilized mAb at 2-8° C. mAb 3 formulations using different buffering systems and at different pHs were tested in stability studies comprised of freeze-thaw cycling, agitation studies, and short-term (12 weeks) incubation at stress, accelerated and intended storage conditions of 40° C, 25° C, 5° C, -30° C, and -80 ° C. In these experiments, aggregation and chemical degradation of the protein were evaluated in addition to particle formation (visual inspection, light obscuration-based particle counting, and micro-flow imaging).

[0103] Based on results from formulation development studies, a formulation containing 10 mM L-histidine-HCl, 8 % w/v sucrose, and 0.05 % w/v polysorbate 80, pH 6.2 was chosen.

Lyophilization Process Development

[0104] A lyophilization process was developed for mAb 3 to ensure that the formulated antibody had acceptable stability and cake attributes following lyophilization. Process parameters of the lyophilization cycle (drying temperatures, chamber pressure, etc.) were determined based on a number of development runs using a lab scale lyophilizer (Genesis EL35 manufactured by SP Scientific). The robustness of the cycle was established by conducting a series of lyophilization runs with controlled excursions in freezing rate, drying temperatures and chamber vacuum pressure. The stability studies on lyophilized formulated antibody comprised of short-term (12 weeks) incubation at stress, accelerated and intended storage conditions of 40° C, 25° C, and 5° C. In these experiments, lyophilized powder attributes (cake appearance, reconstitution time, oxygen headspace, moisture content) were evaluated in addition to aggregation, chemical degradation, and particle formation. Based on formulation and lyophilization development studies, the composition of mAb 3 and was selected as 50 mg/mL mAb 3, 10 mM L-histidine-HCl, 8% w/v sucrose, 0.05% w/v polysorbate 80, and at a pH 6.2. The formulated antibody is stored at ≤ -30° C and lyophilized formulated antibody is stored at 2 - 8° C.

CLAIMS

We claim:

1. An antibody that specifically binds human CD38, wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

2. A pharmaceutical composition comprising a formulated antibody, wherein the formulated antibody comprises an antibody, sucrose, L-histidine, and polysorbate 80 (PS80), wherein the antibody specifically binds human CD38 and is present at a concentration of 50 mg/mL, the sucrose is present at a concentration of 8 % (w/v), the L-histidine is present at a concentration of 10 mM, and the PS80 is present at a concentration of 0.05 % (v/v), wherein the formulation has a pH of 6.2, and wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

3. A unit dosage form of a formulated antibody that specifically binds human CD38, wherein the formulated antibody comprises 215 mg of the antibody, 6.21 mg L-histidine, 344 mg sucrose, and 2.15 mg polysorbate 80, wherein the formulated antibody is lyophilized, and wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

4. The pharmaceutical composition of claim 2 or unit dosage form of claim 3, wherein the formulated antibody is lyophilized.

5. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-4, wherein amino acid at position 1 of the HC amino acid sequence is pyroglutamine.

6. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-5 wherein the antibody has an isoelectric point (pI) of 5.8 to 9.0 when measured by capillary isoelectric focusing (cIEF).

7. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-6, wherein the antibody comprises a main charge variant and at least one acidic charge variant and wherein the antibody has at least one of

a) the HC of the at least one charge variant comprises at least one deamidated asparagine selected from the group consisting of N289, N318, N387, and N392 as numbered according to SEQ ID NO: 1, or

b) the antibody comprises a main charge variant and least one acidic charge variant, wherein the main charge variant comprises at least 71% of the antibody and the acidic charge variant comprises no more than 30% of the antibody, or

c) the main charge variant has an isoelectric point (pI) of 7.5 and wherein the one or more acidic charge variant has pI of 5.8 when measured by capillary isoelectric focusing (cIEF).

8. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-7, wherein the antibody comprises at least one basic charge variant, and wherein the antibody has at least one of

a) the basic charge variant comprises no more than 4% of the antibody, or

b) the one or more basic charge variants has a pI of 9.0 when measured by capillary isoelectric focusing (cIEF).

9. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-8, wherein the antibody

a) is capable of killing a CD38-expressing cell by Antibody Dependent Cellular Cytotoxicity (ADCC), wherein the cell has a CD38 receptor density of ≤13,000 CD38 sites on the surface of the cell in the presence of Natural Killer cells expressing the 158F, 158V, or both 158F and 158V variants CD16a (FcγRIIIa), and/or

b) binds CD16a (FcγRIIIa) having phenylalanine at amino acid position 158 (158F) with a K_D of 59 nM as measured by Surface Plasmon Resonance (SPR) and wherein the antibody binds CD16a having valine at amino acid position 158 (158V) with a K_D of 75 nM as measured by SPR, and/or

c) binds CD16a (FcγRIIIa) having phenylalanine at amino acid position 158 (158F) with a K_D of 96 nM as measured by binding to FcγRIIIa 158F-expressing HEK cells and wherein the antibody binds FcγRIIIa having valine at amino acid position 158 (158V) with a K_D of 40 nM as measured by binding to FcγRIIIa 158V-expressing HEK cells, and/or

d) binds CD32a (FcγRIIa) having arginine at amino acid position 131 (131R) with a K_D of 94 nM as measured by binding to FcγRIIa 131R-expressing HEK cells and wherein the antibody binds FcγRIIa having histidine at amino acid position 131 (131H) with a K_D of 222 nM as measured by binding to FcγRIIa 131H-expressing HEK cells.

10. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-8, wherein

a) the antibody is capable of killing a CD38-expressing cell by ADCC wherein the cell has a CD38 receptor density of >400,000 on the surface of the cell, and is capable of killing a CD38-expressing cell by ADCC wherein the cell has a CD38 receptor density of ≥100,000 on the surface of the cell, and is capable of killing a CD38-expressing cell by ADCC wherein the cell has a CD38 receptor density of ≤13,000 on the surface of the cell, in the presence of Natural Killer cells expressing the 158F, 158V, or both 158F and 158V variants of CD16a (FcγRIIIa) and/or

b) the antibody is capable of killing a CD38-expressing cell by Antibody Dependent Cellular Phagocytosis (ADCP) in the presence of human peripheral blood mononuclear cells (PMBCs), wherein the cell has a CD38 receptor density of >400,000 on the surface of the cell.

11. The antibody, pharmaceutical composition, or unit dosage form of claim 9 or 10, wherein

a) in the presence of Natural Killer cells expressing the higher affinity variant (158V) of CD16a (FcγRIIIa), the antibody is capable of killing KMS-12BM cells by ADCC with an EC 50 of about 0.6 ng/mL and/or

b) in the presence of Natural Killer cells expressing the lower affinity variant (158F) of CD16a (FcγRIIIa), the antibody is capable of killing KMS-12BM cells by ADCC with an EC 50 of about 0.65 ng/mL and/or

c) in the presence of Natural Killer cells expressing the higher affinity variant (158V) of CD16a (FcγRIIIa), the antibody is capable of killing RPMI-8226 cells by ADCC with an EC 50 of about 0.09 ng/mL and/or

d) in the presence of Natural Killer cells expressing the lower affinity variant (158F) of CD16a (FcγRIIIa), the antibody is capable of killing RPMI-8226 cells by ADCC with an EC 50 of about 0.09 ng/mL and/or

e) in the presence of Natural Killer cells expressing the higher affinity variant (158V) of CD16a (FcγRIIIa), the antibody is capable of killing MOLP-8 cells by ADCC with an EC 50 of about 0.14 ng/mL and/or

f) in the presence of Natural Killer cells expressing the lower affinity variant (158F) of CD16a (FcγRIIIa), the antibody is capable of killing MOLP-8 cells by ADCC with an EC 50 of about 0.28 ng/mL.

12. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 9-11, wherein in the presence of human PMBCs expressing the higher affinity variant (158V) of CD16a (FcγRIIIa), the antibody is capable of killing MOLP-8 cells by ADCP with an EC 50 of about 31.87 ng/mL.

13. The antibody, pharmaceutical composition, or unit dosage form of any one of claims 1-12, wherein the antibody is capable of improving survival of mice in a murine tumor model, wherein mice in the model express human CD16a (158F), and wherein the mice have been injected with 500,000 EL4 cells expressing human CD38.

14. A process for preparing pharmaceutical composition comprising an antibody that specifically binds human CD38, wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, the method comprising the steps of:

i) expressing the antibody in a cell culture;

ii) subjecting the antibody to at least one purification step selected from the group consisting of chromatography and ultrafiltration to produce a purified antibody solution; and

iii) adjusting the purified antibody solution to produce a formulated antibody comprising

- the purified antibody at a concentration of 50 mg/mL,
- sucrose at a concentration of 8 % w/v,
- L-histidine at a concentration of 10 mM, and
- polysorbate 80 at a concentration of 0.05 % v/v,

wherein the formulated antibody has a pH of 6.2; and

iv) lyophilizing the formulated antibody,

thereby preparing the pharmaceutical composition.

15. A method for preparing a reconstituted formulated antibody, comprising

a) providing a lyophilized antibody formulation comprising sucrose, L-histidine, polysorbate 80, and an antibody that specifically binds human CD38; and

b) reconstituting the lyophilized antibody formulation in a diluent, wherein the reconstituted antibody formulation comprises

- the antibody at a concentration of 50 mg/mL,
- sucrose at a concentration of 8 % w/v,
- L-Histidine at a concentration of 10 mM, and
- polysorbate 80 at a concentration of 0.05 % v/v,

wherein the reconstituted antibody formulation has a pH of 6.2;

wherein the antibody comprises

i) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

ii) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

iii) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

iv) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

v) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

vi) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

vii) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

16. A method of treating a patient in need thereof, comprising administering to the patient one or more doses of an antibody that specifically binds human CD38, wherein the patient has a disease that comprises CD38-expessing cells, wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

17. An antibody for use in treating a disease that comprises CD38-expessing cells, wherein the antibody specifically binds human CD38, wherein the antibody comprises

a) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 2 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

b) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 3 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

c) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 4 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 7, or

d) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

e) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 5 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9, or

f) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 8, or

g) a heavy chain (HC) having an amino acid sequence of SEQ ID NO: 6 and a light chain (LC) having an amino acid sequence of SEQ ID NO: 9.

18. The method of claim 16 or the antibody for use of claim 17, wherein the disease is of hematological origin.

19. The method or antibody for use of claim 18, wherein the disease of hematological origin is selected from the group consisting of: amyloidosis, non-Hodgkin's lymphoma (NHL), Waldenstrom's disease, multiple myeloma (MM), acute lymphocytic leukemia (ALL), and acute myelogenous leukemia (AML).

20 The method or antibody for use of any one of claims 16-19, wherein the one or more doses of antibody is provided as a lyophilized antibody formulation and wherein prior to administration, the lyophilized antibody formulation is reconstituted forming a reconstituted antibody formulation such that the reconstituted antibody formulation has a pH of 6.2 and comprises the antibody at a concentration of 50 mg/mL in a liquid comprising 10 mM L-histidine, 8 % (w/v) sucrose, and 0.05 % (v/v) polysorbate 80.

21. The method or antibody for use of any one of claims 16-20, wherein the antibody is administered at a dose of 20 mg/kg, 10 mg/kg, 5 mg/kg, 3 mg/kg, or 1.5 mg/kg.

22. The method or antibody for use of any one of claims 16-21, wherein the pharmaceutical composition comprises a formulated antibody, wherein the formulated antibody comprises the antibody present at a concentration of 50 mg/mL, sucrose at a concentration of 8 % (w/v), L-histidine at a concentration of 10 mM, and polysorbate 80 (PS80) at a concentration of 0.05 % (v/v), wherein the formulated antibody has a pH of 6.2.

23. The method or antibody for use of any one of claims 16-22, wherein the antibody has an isoelectric point (pI) of 5.8 to 9.0 when measured by capillary isoelectric focusing (cIEF).

24. The method or antibody for use of any one of claims 16-23, wherein amino acid at position 1 of the HC amino acid sequence is pyroglutamine.

25. The method or antibody for use of any one of claims 16-25, wherein the antibody comprises a main charge variant and at least one acidic charge variant and wherein the antibody has at least one of

a) the HC of the at least one charge variant comprises at least one deamidated asparagine selected from the group consisting of N289, N318, N387, and N392 as numbered according to SEQ ID NO: 1, or

b) the antibody comprises a main charge variant and least one acidic charge variant, wherein the main charge variant comprises at least 71% of the antibody and the acidic charge variant comprises no more than 30% of the antibody, or

c) the main charge variant has an isoelectric point (pI) of 7.5 and wherein the one or more acidic charge variant has pI of 5.8 when measured by capillary isoelectric focusing (cIEF).

26. The method or use of any one of claims 16-25, wherein the antibody comprises at least one basic charge variant, and wherein the antibody has at least one of

a) the basic charge variant comprises no more than 4% of the antibody, or

b) the one or more basic charge variants has a pI of 9.0 when measured by capillary isoelectric focusing (cIEF).

FIG. 1

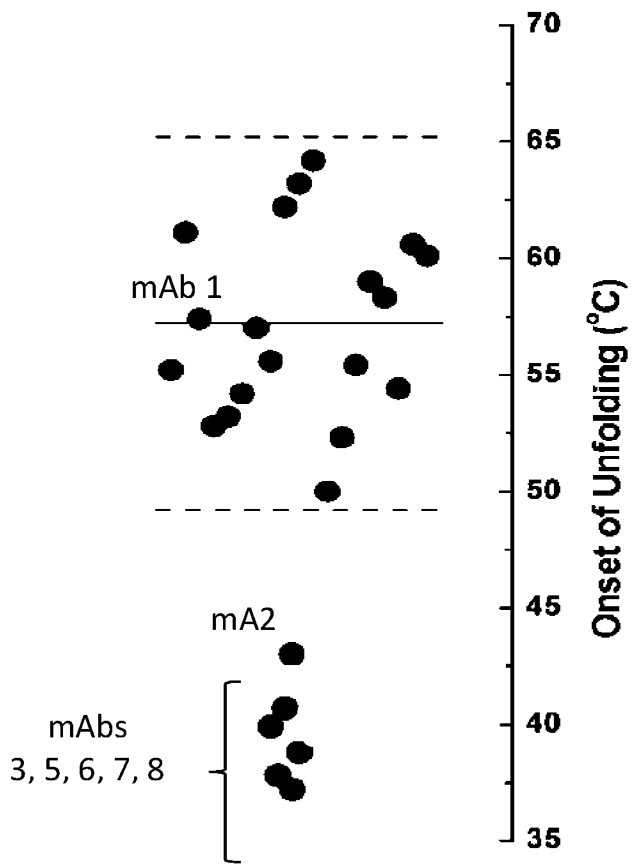
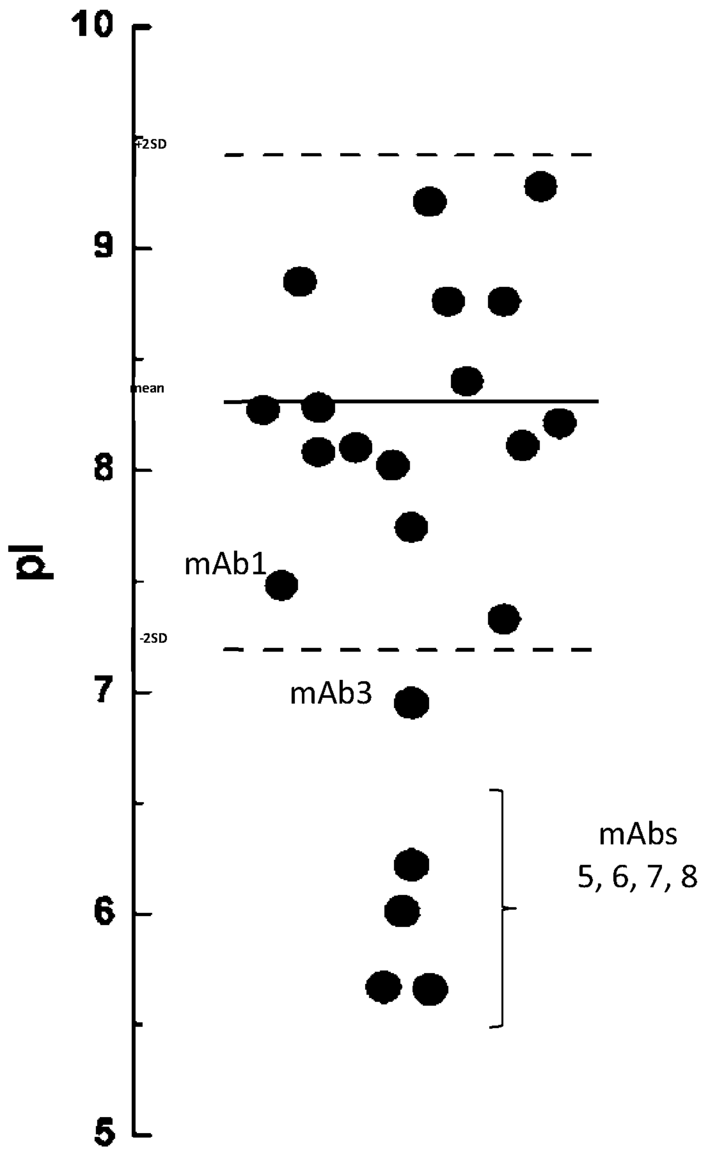


FIG 2.



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2020/029531

A. CLASSIFICATION OF SUBJECT MATTER
INV. A61K39/395 C07K16/28 A61P35/00
ADD. A61K39/00

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)
A61K C07K A61P

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, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 2008/047242 A2 (SANOFI AVENTIS [FR]; PARK PETER U [US] ET AL.) 24 April 2008 (2008-04-24) sequences 66,72	1-26
Y	XINHUA WANG ET AL: "IgG Fc engineering to modulate antibody effector functions", PROTEIN & CELL, vol. 9, no. 1, 6 October 2017 (2017-10-06), pages 63-73, XP055457296, Beijing, CN ISSN: 1674-800X, DOI: 10.1007/s13238-017-0473-8 table 1	1-26

-/--

[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
4 August 2020	14/08/2020

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	Wagner, René

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	DILILLO DAVID J ET AL: "Differential Fc-Receptor Engagement Drives an Anti-tumor Vaccinal Effect", CELL, ELSEVIER, AMSTERDAM, NL, vol. 161, no. 5, 11 May 2015 (2015-05-11), pages 1035-1045, XP029129128, ISSN: 0092-8674, DOI: 10.1016/J.CELL.2015.04.016 page 1309 -----	1-26
Y	SEAN H GAO ET AL: "Monoclonal antibody humanness score and its applications", BMC BIOTECHNOLOGY, BIOMED CENTRAL LTD. LONDON, GB, vol. 13, no. 1, 5 July 2013 (2013-07-05), page 55, XP021156542, ISSN: 1472-6750, DOI: 10.1186/1472-6750-13-55 page 9, left-hand column -----	1-26
A	WANG W ET AL: "ANTIBODY STRUCTURE, INSTABILITY, AND FORMULATION", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN CHEMICAL SOCIETY AND AMERICAN PHARMACEUTICAL ASSOCIATION, US, vol. 96, no. 1, 1 January 2007 (2007-01-01), pages 1-26, XP009084505, ISSN: 0022-3549, DOI: 10.1002/JPS.20727 page 14 -----	1-26
A	SHIRE STEVEN J ET AL: "Challenges in the development of high protein concentration formulations", JOURNAL OF PHARMACEUTICAL SCIENCES, AMERICAN CHEMICAL SOCIETY AND AMERICAN PHARMACEUTICAL ASSOCIATION, US, vol. 93, no. 6, 1 June 2004 (2004-06-01), pages 1390-1402, XP009108986, ISSN: 0022-3549, DOI: 10.1002/JPS.20079 figure 2 -----	1-26
A	DAUGHERTY ANN L ET AL: "CHAPTER 8: Formulation and delivery issues for monoclonal antibody therapeutics", 1 January 2010 (2010-01-01), CURRENT TRENDS IN MONOCLONAL ANTIBODY DEVELOPMENT AND MANUFACTURING, SPRINGER, US, PAGE(S) 103 - 129, XP009180430, ISBN: 978-0-387-76642-3 chapter 4 ----- -/--	1-26

1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LAZAR GREG A ET AL: "Engineered antibody Fc variants with enhanced effector function", PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, NATIONAL ACADEMY OF SCIENCES, vol. 103, no. 11, 14 March 2006 (2006-03-14), pages 4005-4010, XP002590010, ISSN: 0027-8424, DOI: 10.1073/PNAS.0508123103 figure 3 -----	1-26
A	BAEK YOUNGBIN ET AL: "Effects of Histidine and Sucrose on the Biophysical Properties of a Monoclonal Antibody", PHARMACEUTICAL RESEARCH, SPRINGER NEW YORK LLC, US, vol. 34, no. 3, 29 December 2016 (2016-12-29), pages 629-639, XP036145509, ISSN: 0724-8741, DOI: 10.1007/S11095-016-2092-0 [retrieved on 2016-12-29] figure 9 -----	1-26
A	FENG HE ET AL: "Effect of Sugar Molecules on the Viscosity of High Concentration Monoclonal Antibody Solutions", PHARMACEUTICAL RESEARCH, KLUWER ACADEMIC PUBLISHERS-PLENUM PUBLISHERS, NL, vol. 28, no. 7, 15 May 2011 (2011-05-15), pages 1552-1560, XP019912569, ISSN: 1573-904X, DOI: 10.1007/S11095-011-0388-7 figure 2 -----	1-26
A	SHUJING WANG ET AL: "Viscosity-Lowering Effect of Amino Acids and Salts on Highly Concentrated Solutions of Two IgG1 Monoclonal Antibodies", MOLECULAR PHARMACEUTICS, vol. 12, no. 12, 7 December 2015 (2015-12-07), pages 4478-4487, XP055385371, US ISSN: 1543-8384, DOI: 10.1021/acs.molpharmaceut.5b00643 the whole document -----	1-26

1

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/029531

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2008047242 A2	24-04-2008	AR 063488 A1	28-01-2009
		AU 2007311511 A1	24-04-2008
		BR PI0718130 A2	05-11-2013
		CA 2663209 A1	24-04-2008
		CA 2989609 A1	24-04-2008
		CL 2007002995 A1	16-05-2008
		CL 2012002012 A1	05-11-2012
		CL 2017001580 A1	02-03-2018
		CN 101616933 A	30-12-2009
		CO 6190555 A2	19-08-2010
		CR 10679 A	24-06-2009
		DK 2076540 T3	13-05-2019
		DO P2017000177 A	15-09-2017
		EA 200970393 A1	30-12-2009
		EP 1914242 A1	23-04-2008
		EP 2076540 A2	08-07-2009
		EP 3498735 A1	19-06-2019
		ES 2726754 T3	09-10-2019
		GT 200900079 A	09-07-2010
		HK 1139956 A1	29-04-2016
		HR P20190742 T1	14-06-2019
		HU E043351 T2	28-08-2019
		IL 197217 A	31-12-2019
		JP 5607364 B2	15-10-2014
		JP 6121366 B2	26-04-2017
		JP 2010506582 A	04-03-2010
		JP 2014221761 A	27-11-2014
		KR 20090078330 A	17-07-2009
		KR 20140105035 A	29-08-2014
		KR 20150023010 A	04-03-2015
		KR 20150119976 A	26-10-2015
		KR 20170129960 A	27-11-2017
		LT 2076540 T	10-06-2019
		MA 30895 B1	02-11-2009
		ME P10609 A	20-12-2011
		MY 159374 A	30-12-2016
		NZ 575188 A	27-07-2012
		PE 20081171 A1	26-09-2008
		PL 2076540 T3	30-08-2019
		PT 2076540 T	06-06-2019
		SG 173345 A1	29-08-2011
		SG 174777 A1	28-10-2011
		SG 10201501733Q A	29-04-2015
		SI 2076540 T1	28-06-2019
		SV 2009003203 A	27-10-2009
		TN 2009000058 A1	19-08-2010
		TW 200823236 A	01-06-2008
		TW 201518322 A	16-05-2015
		TW 201630938 A	01-09-2016
		TW 201734053 A	01-10-2017
		UA 114879 C2	28-08-2017
		US 2009304710 A1	10-12-2009
		US 2011262454 A1	27-10-2011
		US 2012156218 A1	21-06-2012
		US 2017343550 A1	30-11-2017
		UY 30655 A1	31-05-2008
		WO 2008047242 A2	24-04-2008
		ZA 200901395 B	30-06-2010

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2020/029531

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
--			

Form PCT/ISA/210 (patent family annex) (April 2005)